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TABLE OF CONTENTS

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- APR 2024 30 — Abstract submission close
- JUNE 2024 07 — Abstracts Result Announcement
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JAFES

A Journal's Hormonal Balance

Let us consider that this generation of JAFES is in adolescence: we must help it maintain a balance of hormones that, over time, affect the Journal's many processes, including mood, growth, development, metabolism and reproduction. The Journal needs to function well through its organs and systems: foremost, our readers including health care professionals and their patients; equally, our authors and their institutions; and finally, our member Societies.

Our *readers* are served well by the open-access format of the Journal, bringing closer to them the Southeast Asia-specific research data and findings on endocrine-related conditions.

Our *authors* are empowered by the supportive editorial policies and processes that aim for clear, relevant and timely publications.

Our *member Societies* are enhanced in the pursuit of their missions.

Thirteen years young and growing, what then concerns the JAFES of this generation?

In the recent years since 2011, readers have benefitted by more than 2.5 million views and downloads from our JAFES website.

With indexing on several platforms—initially on Scopus in 2017, Web of Science in 2019, PubMed Central in 2020 for articles since 2017, and most recently on PubMed/MEDLINE since December 2022—our authors and other researchers now have more than 240 articles contributed through JAFES to the global body of knowledge. And the article submissions keep coming in in greater numbers.

Through these 13 years, all seven member Societies of AFES have demonstrated that supporting research publication is one mission that they share, and one service that they share with their individual members. Annual funds have been consistently contributed by the Societies. More than 433 JAFES articles have been published whose author or authors are Society members.

Now, then, is time for JAFES to depend on other funds—in effect other hormones—for further growth and development. It is our editorial team's considered opinion that a small dose, a modest amount, of article processing charge, is reasonably indicated. The amount must remain tolerable by Southeast Asian authors and their research fund providers.

This way over a course of another six years, give or take, JAFES should be able to depend less on funds contributed by the member Societies and depend more on contributions by the authors themselves with their research funders.

We look to enhanced hormonal interactions to preserve balance through maturity. We look to greater impact and relevance, stronger impetus for policy change and closer collaboration within the region.

Let me take this opportunity to invite colleagues across the region to join in the celebration of the gift that keeps giving. *Mabuhay!*

Elizabeth Paz-Pacheco
Editor-in-Chief

https://doi.org/10.15605/jafes.038.02.01

AFES 2023
22nd ASEAN FEDERATION OF ENDOCRINE SOCIETIES CONGRESS

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16 NOVEMBER 2023
16:15-16:45 HRS.
Ballroom 1, Queen Sirikit National Convention Center
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WE ARE CORDIALLY INVITING YOU TO ATTEND

THE JAFES SYMPOSIUM FEATURING A BRIEF TALK ON AI AND ITS EMERGING IMPACT ON SCHOLARLY PUBLICATION.

16:15-16:20 HRS.

Dr. Elizabeth Paz-Pacheco
Editor-in-Chief
Welcome remarks
(JAFES milestones and accomplishments)

16:20-16:30 HRS.

Dr. Cecilia A. Jimeno
Vice Editor-in-Chief
Topic: Emerging AI Issue in Scientific Publication

16:30-16:40 HRS.

Dr. Gabriel V. Jasul, Jr.
Associate Editor
Recognition of JAFES Top Authors and Reviewers

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Journal of the ASEAN Federation of Endocrine Societies

ORIGINAL ARTICLE

Characteristics of Children with Newly Diagnosed Type 1 Diabetes Mellitus in Brunei Darussalam

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Abstract

Objective. This study aims to characterize the presentation, biochemical status of children with T1DM at diagnosis, the type of subcutaneous insulin regimens initiated, and to determine the incidence of T1DM in Bruneian children aged 18 years and younger.

Methodology. A retrospective electronic and paper medical chart review was performed on patients aged 18 years and younger diagnosed with T1DM from 2013 to 2018 in Brunei Darussalam.

Results. A total of 31 children with a mean age of 10.2 ± 3.6 years old were diagnosed with T1DM, of which 66.7% presented with diabetic ketoacidosis (DKA), a majority in severe DKA with an intercurrent illness (p = 0.021). The mean HbA1c was 13.6 ± 2.7% with a mean serum glucose of 37.0 ± 14.9 mmol/L at diagnosis. In the majority of the children (67.7%), multiple daily injections of subcutaneous insulin were initiated. The incidence of T1DM in children aged 18 years and younger was 4.9 per 100,000 for the year 2018.

Conclusions. The majority of the patients in this study presented with severe DKA with an intercurrent illness. This highlights the importance of childhood T1DM awareness among the public and healthcare providers. The incidence of childhood T1DM in Brunei Darussalam is similar to other countries in the Asian region, being relatively low, compared to the rest of the world.

Key words: type 1 diabetes mellitus, diabetic ketoacidosis, pediatric

INTRODUCTION

Globally, type 1 diabetes mellitus (T1DM) is the most common type of diabetes affecting children and adolescents.[1,2] Symptoms of T1DM in children are often vague and T1DM may initially present with an intercurrent illness, which renders arriving at an accurate diagnosis challenging.[2-6] Several studies have shown that the mean duration of symptoms prior to diagnosis is over 2 weeks with a significant number of children experiencing delay in diagnosis or misdiagnosis and only one in five is diagnosed correctly during their first encounter with a physician.[7,8] Often, there is a concurrent illness noted in young children at diagnosis, causing an acute metabolic decompensation leading to diabetic ketoacidosis (DKA), which has immediate life-threatening implications and is associated with poorer long-term diabetic control.[9-11] Hence, early identification is important in preventing the morbidity and potential mortality associated with new-onset diabetes in this vulnerable age group.[9]

Multiple epidemiological studies have demonstrated that the incidence of T1DM is on the rise around the world;[3-5] however, there are currently no published data on the incidence of pediatric T1DM in Brunei Darussalam. In the Asian region, the incidence of T1DM is relatively low compared to the rest of the world, at approximately 2 to 5 per 100,000 person-years.[12,13] In neighboring Singapore, the incidence of Type 1 diabetes in children aged 0-12 years is 2.46 per 100, 000 children.[13] Meanwhile, according to the Malaysian Diabetes in Children and Adolescents Registry, 71.8% of children with diabetes mellitus in Malaysia under the age of 20 years had T1DM with more than half of the patients (58.3%) presenting with DKA at diagnosis.[14]

The objectives of this study are to characterize the presentation, family history, and biochemical status at the time of diagnosis of T1DM, and to determine the incidence of T1DM in Bruneian children. Additionally, the study aims to determine the type of subcutaneous insulin regimens initiated for these children.

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METHODOLOGY

All children aged 18 years and younger who were diagnosed with new-onset T1DM from 1st January 2013 to 31st December 2018 in Brunei Darussalam were identified through an extensive computerized search from the complete list of the ICD-10 codes for T1DM (E10.X) in Bru-HIMS (Brunei Darussalam Healthcare Information and Management Systems) and a nationwide pediatric clinic database of pediatric patients on follow-up for diabetes mellitus in all government hospitals in Brunei Darussalam. The extensive list of ICD-10 codes is included in Appendix 1. Bru-HIMS is an electronic health record system in which all Bruneian patients are mandated to register where their health information are collected under one patient record. This was officially launched nationwide on September 11, 2012. It is a nationwide population-based healthcare database and the capture rate is almost 100% as it is used in all government hospitals, outpatient services, treatment centers and clinics in Brunei Darussalam. Bru-HIMS is not available though in private clinics and hospitals. Still, all children with T1DM are eventually referred to government hospitals for management and continuation of care. This is because Brunei is a small country, and there is only one main tertiary hospital that can provided appropriate intensive care and diabetes care for pediatric patients. Hence, the capture rate for all children diagnosed with T1DM is high through Bru-HIMS.

A systematic, retrospective electronic medical chart review was performed for all children aged 18 years and younger with new-onset T1DM between 2013 and 2018. Data extracted from paper and electronic patient records included demographic, clinical and biochemical details. Information including symptoms of weight loss, polyuria, polydipsia, nocturia and presence of any intercurrent illness (such as viral gastroenteritis or cellulitis) at the time of diagnosis of T1DM were collected. Having a first-degree relative with T1DM or T2DM (type 2 diabetes mellitus) was considered as a positive family history. DKA was defined as hyperglycemia with serum glucose ≥11.1 mmol/L, venous pH <7.30, and/or serum bicarbonate <15 mmol/L in the presence of ketones.[15,16] Furthermore, the severity of the DKA was categorized as "Mild": Venous pH <7.3 or serum bicarbonate <15.0 mmol/L, "Moderate": Venous pH <7.2, serum bicarbonate <10.0 mmol/L, or "Severe" Venous pH <7.1, serum bicarbonate <5.0 mmol/L.[15,16] In addition, the type of subcutaneous insulin regimen prescribed subsequently: twice daily insulin (intermediate acting with short acting insulin) or multiple daily injections (long-acting insulin once daily with pre-meal short acting insulin three times per day), were also recorded.

All patient information were de-identified and recorded into a password-protected research database. During data extraction, each case was examined carefully to ensure that patients who were older than 18 years at the time of diagnosis or who had other types of diabetes mellitus besides T1DM such as type 2 diabetes mellitus, diabetes associated syndrome such as DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness) or other causes including thalassemia -related diabetes or cystic fibrosis-related diabetes were excluded from the study. Ethical approval was obtained from the Brunei Darussalam Ministry of Health Research and Ethical Committee prior to the commencement of the study.

For statistical analysis, the data from this study were analyzed using Statistical Package for Social Sciences Program (SPSS, Version 20, IBM, Chicago, Illinois, USA). Frequency data were presented as mean ± standard deviation. Laboratory data were stratified by metabolic status (DKA or non-DKA at presentation) with mean ± standard deviation. Fisher's exact test was carried out for variables with less than 5 expected entries in more than 20% of the cells. A p-value of <0.05 was used as a cut-off for all tests to ascertain statistical significance.

The incidence of T1DM in children aged 18 years and younger was calculated by dividing the total number of new T1DM cases by the population consisting of 18 years and under in Brunei Darussalam for that year. We acquired the information on the number of children aged 18 years and younger in Brunei Darussalam through the Brunei Department of Economic Planning and Development.[17]

RESULTS

A total of 31 children were newly diagnosed with T1DM from 2013 to 2018 in Brunei Darussalam. The mean age at the time of diagnosis of T1DM was 10.2 ± 3.6 years with a near equal distribution between males and females. In terms of ethnicity, the largest proportion of patients were Malays comprising 74.2% of the study population, as it is the race of the majority of the population in the country. None of the children had a first-degree relative with T1DM, though 19.4% did have a first-degree relative with T2DM.

The children predominantly presented with classic osmotic symptoms of T1DM including polyuria in 22 patients (93.1%), polydipsia in 26 patients (92.9%) and nocturia in 20 patients (90.0%). Weight loss was also frequently reported in 72.4% of the children in our study.

A total of 21 patients (66.7%) patients presented with DKA. Majority of them, 71.4%, were in severe DKA, 23.8% in moderate DKA and the remaining 4.8% presented with mild DKA. Of significance, a larger number of children in the DKA group also had an intercurrent illness such as viral gastroenteritis and cellulitis at the time of diagnosis compared to the non-DKA group (61% versus 30%, p = 0.02). None of the children in this study had cerebral edema and there were no mortalities from DKA or T1DM.

The mean serum glucose at diagnosis was 37.0 ± 14.9 mmol/L with mean HbA1c of 13.6 ± 2.7 % though both were not statistically different between the DKA and non-DKA group. Twenty-two patients in our study had low

C-peptide levels (<370.0 pmol/L) and majority had at least the presence of one auto-antibody at presentation. As part of our routine management for newly diagnosed patients with DKA, only anti-islet cell antibodies and anti- glutamic acid decarboxylase were tested. Furthermore, 42.6% of the children in our study had abnormal thyroid function tests at the time of diagnosis (Table 1).

Different subcutaneous insulin regimens were prescribed, with 21 patients (67.7%) on multiple daily insulin injections while the remaining 10 patients (32.3%) were on the twice daily insulin injections. No patients were commenced on insulin pump therapy.

Table 1. Baseline demographics and biochemistry of all children aged 18 years and younger diagnosed with new-onset Type 1 Diabetes Mellitus from 1st January 2013 to 31st December 2018 in Brunei Darussalam

Baseline Characteristics	Mean ± SD, n (%)
Age at diagnosis in years, mean ± SD	10.2 ± 3.6
Sex, n (%)	
Male	14/30 (47.0%)
Female	14/30 (47.0%)
Race, n (%)	
Malay	23/31 (74.2%)
Non-Malays	8/31 (25.8%)
First degree relatives with T1DM, n (%)	0/30 (0%)
First degree relatives with T2DM, n (%)	6/31 (19.4%)
Symptoms at diagnosis, n (%)	
Weight loss	21/29 (72.4%)
Polyuria	27/29 (93.1%)
Polydipsia	26/28 (92.9%)
Nocturia	20/22 (90.9%)
Intercurrent illness	19/31 (61.3%)
Diabetic Ketoacidosis at presentation	21/31 (67.7%)
Serum glucose at diagnosis (mmol/L), mean ± SD	37.0 ± 14.9
HbA1c at diagnosis (%), mean ± SD	13.6 ± 2.7
Low C-peptide <370.0 pmol/L at presentation	22/29 (75.9%)
Presence of at least one autoantibody, n (%)	19/26 (73.1%)
One positive antibody	9/26 (34.6%)
Two positive antibodies	10/26 (38.5%)
Glutamic acid decarboxylase (GAD) positivity, n (%)	16/26 (61.5%)
Insulinoma-antigen 2 (IA2) positivity, n (%)	13/26 (38.5%)
Abnormal Thyroid function test, n (%)	12/26 (46.2%)

Published population data by the Brunei Department of Economic Planning and Development for children under the age of 18 years and under was only available for the year of 2018.[17] Hence, we were only able to calculate the incidence of T1DM in pediatric patients for that particular year. In 2018, the population in Brunei Darussalam was 442,400, with 27.5% of the population being aged 18 years and under.[17] There were 6 pediatric patients who were Bruneian or permanent residents of Brunei Darussalam that were newly diagnosed with T1DM for the year of 2018. Utilizing this available information, the incidence of T1DM in children aged 18 years and younger was calculated to be 4.9 per 100,000 for the year 2018.

DISCUSSION

This is the first population-based study carried out in children with T1DM in Brunei Darussalam examining the clinical and biochemical characteristics at diagnosis. During the 6-year study period, a total of 31 children aged 18 years and younger were diagnosed with T1DM. More than half of the patients in this study (66.7%) presented with DKA, predominantly with severe DKA (Table 2). This highlights the importance of early identification of children with T1DM, ideally before the development of DKA as this acute condition is associated with high morbidity and mortality. The presence of DKA at initial presentation in our cohort is comparatively higher than that reported from developed countries such as Sweden (12.8%), Finland (19.0%) and UK (25.0%) where prevalence rates of T1DM are higher.[18-21] This may be attributed to different racial and environmental factors but may also reflect the lack of public awareness of diabetic symptoms in children among the Bruneian population.

Comparable to other studies,[6-10] the children in our study also predominantly presented with the classic osmotic symptoms of T1DM such as polyuria, polydipsia and nocturia. Of significance, majority who presented with DKA also had an intercurrent illness at the time of diagnosis, which likely contributed to the acute metabolic decompensation that can lead to immediate, life-threatening

Table 2. Comparison between †DKA and Non-†DKA group

	†DKA (n = 21)	Non-†DKA (n = 10)	*p*
Sex, n (%)			
Male	9/21 (42.9%)	5/10 (50.0%)	
Female	12/21 (57.1%)	5/10 (50.0%)	1.00
Race, n (%)			
Malay	17/21 (80.9%)	6/10 (60.0%)	
Non-Malays	4/21 (19.1%)	4/10 (40.0%)	0.38
First degree relatives with T1DM, n (%)	0/20 (0%)	0/10 (0%)	-
First degree relatives with T2DM, n (%)	4/21 (19.0%)	2/10 (20.0%)	1.00
Low C-peptide levels (<370 pmol/L)	18/20 (90.0%)	5/8 (62.5%)	0.123
Presence of intercurrent illness	16/21 (61.3%)	3/10 (30.0%)	0.02*
Presence of at least one autoantibody, % (n)	12 /17 (70.6%)	7/9 (77.7%)	1.00
One positive antibody	4/17 (23.5%)	5/9 (55.6%)	0.19
Two positive antibodies	8/17 (47.1%)	2/9 (22.2%)	0.40

†DKA: Diabetic Ketoacidosis
*A *p*-value of <0.05 was used as a cut-off for all tests of statistical significance. Fisher's exact tests were carried out for variables with less than 5 entries.

complications.[7,8] The intercurrent illnesses detected were cellulitis and viral gastroenteritis that present with vague symptoms such as vomiting or abdominal pain. These illnesses can trigger DKA or mimic the acute presentation of DKA. Thus, symptoms of T1DM may be misinterpreted, leading to delayed diagnosis and significant morbidity. More concerning in the findings of our study was that some patients were already symptomatic with polyuria, polydipsia, nocturia and weight loss for up to 3 months before seeking medical attention. Such delay in pursuing medical care may have serious health implications.

In contrast to western countries where there are T1DM patients who have a positive family history [18-21], none of the children in our study had any first-degree relatives with T1DM. However, 19.4% had a first-degree relative with T2DM, which demonstrates the worrying rising trend in the prevalence of T2DM among the adult Brunei population, mostly as a consequence of sedentary lifestyle and obesity. A recent population survey demonstrated a 28% obesity prevalence and 10% T2DM prevalence in the Brunei adult population.[22]

Patients with T1DM have a higher prevalence of auto-immune diseases compared to patients without diabetes.[5] Nearly half (42.6%) of the children in our study had abnormal thyroid function tests during presentation but none had any subsequent formal diagnosis of auto-immune thyroid disorder on follow-up. This is likely attributed to non-thyroidal illness during the acute presentation.

The "basal-bolus" or the multiple daily insulin injection regimen, which mimics the physiological insulin secretion is the preferred choice for treatment, as majority (67.7%) of patients were commenced on this regimen. No patients were prescribed insulin pump therapy at discharge, due to our relative inexperience with this insulin regimen in the pediatric population, although this service has been provided in adult endocrine clinics since 2013.

The incidence of T1DM in children aged 18 years and younger is 4.9 per 100,000 for the year 2018. To our knowledge, this is the first study looking at the incidence of childhood T1DM in Brunei Darussalam. The worldwide incidence of T1DM is quite variable and the data from our study suggest that the incidence of T1DM in children in Brunei is quite low compared to the countries with the highest incidence namely Finland and Italy (36.5 and 36.8/100,000 per year, respectively).[12,21]

Our study has several limitations. The number of patients in this cohort is small given the population of Brunei Darussalam. It is also a retrospective study done through chart review which relies on the documentation of others; therefore, some clinical details may be missing from the chart. Another limitation of the study is the possibility, albeit remote, of missing data for patients' encounter at private clinics and hospitals as these are not captured in Bru-HIMS.

CONCLUSION

The result from our study demonstrated that a significant proportion of our children with T1DM presented with severe DKA at diagnosis, often accompanied by an intercurrent illness. The common symptoms of T1DM such as polyuria, polydipsia, nocturia and weight loss can be easily recognized by parents and physicians. This will allow early diagnosis, thus avoiding the potentially life-threatening complications associated with delayed presentation. The lower incidence of T1DM in Brunei Darussalam compared to the rest of the world, may have contributed to the lack of awareness about this medical condition among the general Brunei population. In western countries, where the prevalence of T1DM are higher, prevention programs utilizing education sessions and information dissemination via mass media have been demonstrated to be effective in reducing rates of DKA.[23-25] A mass-media campaign carried out in schools and pediatricians' offices over the course of 8 years in the Italian province of Parma have proven to be successful in reducing the incidence of pediatric DKA from 78% to 12.5%.[23] Public awareness campaigns in the UK have also garnered similar success.[25] In conclusion, the authors suggest conducting public awareness campaigns to help educate the public as well as the healthcare providers in Brunei Darussalam on symptoms associated with childhood T1DM to enable the early diagnosis and prevention of DKA.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
CYW: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **AMLY:** Data curation, Writing – review and editing, Supervision; **CFC:** Formal analysis, Data curation, Writing – review and editing, Visualization; **INSS:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – review and editing, Supervision, Project administration.

Author Disclosure
The authors declared no conflict of interest.

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APPENDIX

ICD-10 Codes for Type 1 Diabetes Mellitus available on Bru-HIMS

ICD-10 Codes	Description
E10	Type 1 diabetes mellitus (T1DM)
E10.0	T1DM: With coma
E10.1	T1DM: With ketoacidosis
E10.2	T1DM: With renal complications
E10.3	T1DM: With ophthalmic complications
E10.4	T1DM: With neurological complications
E10.5	T1DM: With peripheral circulatory complications
E10.6	T1DM: With other specified complications
E10.7	T1DM: With multiple complications
E10.8	T1DM: With unspecified complications
E10.9	T1DM: Without complications

ORIGINAL ARTICLE

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Behavioural and Emotional Problems in Malaysian Children and Adolescents with Type 1 Diabetes Mellitus: A Cross-sectional Study in a Single Centre*

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Abstract

Introduction. Type 1 diabetes mellitus (T1DM) is an autoimmune disorder that requires a lifelong treatment regimen which may affect psychosocial development.

Objective. To identify behavioural and emotional problems in children and adolescents with T1DM.

Methodology. A cross-sectional study using the Child Behaviour Check List (CBCL) was conducted among all T1DM patients receiving treatment at the Paediatric Endocrine Unit, Hospital Tunku Azizah Kuala Lumpur, Malaysia.

Results. Forty T1DM patients were included. The mean age of the participants was 12.4 years (SD = 2.69), with 52.5% males, and 75% Malay. The average duration of illness was 4.8 years, 9 were pre-pubertal, while mean HbA1c was 9.4%. Thirty-five percent of the respondents had parent-reported internalizing problems and 17.5% had parent-reported externalizing problems. Those >12 years old had more internalizing problems (p = 0.004) compared to those ≤12 years old. The differences were in the anxious/depressed syndrome subscale (p = 0.001) and withdrawn/depressed syndrome subscale (p = 0.015). There were no statistically significant differences in the 3 main global scores by gender, glycaemic control, duration of illness and pubertal status by univariate analysis.

Conclusion. T1DM patients >12 years old were at higher risk of developing psychosocial difficulties. This highlighted the benefit of screening of behavioural and emotional issues in children and adolescents with T1DM.

Key words: Type 1 Diabetes Mellitus, psychosocial, Child Behaviour Check List, CBCL

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder requiring a lifelong treatment regimen of diet, exercise, and insulin injections to achieve a normal metabolic state.[1] Despite improvements in well-being of T1DM patients attributed to advancement in insulin therapy, the morbidity and mortality remains significant.[1] This may lead to psychosocial complications and possibly affect their glycaemic control. Globally, 1,211,900 children and adolescents <20 years are estimated to have T1DM.[2] It is estimated that around 149,500 children and adolescents are diagnosed each year.[2] In Southeast Asia, it is estimated that there are 25,700 newly diagnosed T1DM in children and adolescents each year.[2]

It is known that internalizing problems such as depressive mood and anxiety are significantly higher in children with T1DM compared to healthy controls.[3-5] In a study of 84 T1DM children aged 6 to 14 years in India, there was a higher prevalence of psychosocial illness (including irritation, depression and anxiety) in the T1DM group compared to the control group (55.95% vs 20%; p<0.0001).[6] Another study from China consisting of 45 T1DM children also showed similar findings, with the T1DM group having significantly higher scores in psycho-social behavioural problems in T1DM group compared to the control group.[7]

Generally, adolescents with diabetes scored lower in social acceptance compared with healthy adolescents.[8] Over time, depressive symptoms and anxiety increased and

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self-worth decreased for female T1DM patients.[8] Hence, psychological problems in children with diabetes necessitate multidisciplinary management and collaboration between paediatricians and mental health professionals. [9]

Glycosylated haemoglobin (HbA1c) reflects the average level of serum glucose for the last 5-8 weeks and it is one of the metabolic control indicators in diabetes. Poor mental health[10-12] and high levels of stress[13,14] can affect metabolic control and impair treatment processes. Depression, along with poor metabolic control, may result in more complications, poorer outcomes, and more frequent hospitalization.[15] Late adolescence has been identified as a period with a higher rate of acute complications and relative mortality risks for individuals with diabetes.[16] Psychological evaluation and intervention are important in the management of T1DM in children and adolescents because diabetes care could be compromised during this period. A study on behavioural and emotional problems in children and adolescents with T1DM has never been done in our centre, and no similar study has been reported in Malaysia. Physician's awareness on the psychological issues surrounding T1DM patients will be beneficial as a more holistic approach in diabetes care can be planned and developed.

The main purpose of this study is to determine behavioural and emotional problems in children and adolescents with T1DM in a single centre in Malaysia. In addition, this study examines the association between unfavourable behavioural and emotional characteristics and other factors such as glycaemic control and pubertal status in T1DM.

METHODOLOGY

Study type and design

A cross-sectional study using a parent self-report questionnaire was conducted in Paediatric Endocrine Unit, Hospital Tunku Azizah which is a tertiary centre in Malaysia. The study was carried out from 29th April 2019 and 15th July 2021. T1DM patients in the age group of 6 to 18 years old were identified and their parents or caretakers were approached to participate in the study. Consent was obtained from parents who were willing to participate in the study.

Inclusion criteria

Universal sampling of all T1DM patients aged between 6 to 18 years receiving treatment at the Paediatric Endocrine Unit in Hospital Tunku Azizah Kuala Lumpur between 29th April 2019 and 15th July 2021.

Exclusion criteria

1. Presence of conditions associated with neurodevelopmental impairment including intellectual disability or learning disability, cerebral palsy, head injury, brain tumours, etc.
2. Syndromic conditions such as Down syndrome.
3. Pre-existing psychiatric illnesses e.g., Major Depressive Disorder, Schizophrenia, Autism.

Study instrument

The Child Behaviour Check List (CBCL)[17] was used in this study. The CBCL, developed by Thomas M. Achenbach, is a parent-reported questionnaire consisting of 118 items used as screening tool to assess behavioural and emotional problems. There are 8 syndrome subscales in CBCL; anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behaviour, and aggressive behaviour. Each subscale score is interpreted based on T-scores and percentile scores. T-scores of <65 (<95th percentile) are considered to be within normal range, T-scores of 65-70 (95-98th percentile) are considered to be in the borderline range, while T-scores >70 (>98th percentile) are considered to be in clinical range. There are also 3 global scores, internalizing problems (consisting of 3 syndrome scales - anxious/depressed, withdrawn/depressed, somatic complaints), externalizing problems (consisting of 2 syndrome scales - rule-breaking behaviour, and aggressive behaviour) and total problems (total score of all 8 syndrome subscales). These global scores are also categorised based on T-scores whereby scores <60 are considered within the normal range; 60-63 are in the borderline range; and >63 are in the abnormal range. The CBCL is used worldwide, has been validated for use and translated into multiple languages. In this study, the Bahasa Malaysia (BM) version was used. An internal validation study for the BM version of CBCL has been conducted locally and the Cronbach's alpha for internal consistency ranged from 0.7 to 0.9.[18]

Demographic, clinical and biochemical data were obtained from medical records. Data obtained such as age, gender, race, pubertal status, onset of diagnosis, and latest HbA1c results to evaluate the age distribution, duration of illness and their glycaemic control. Age was categorized as a binary variable (≤12 years versus >12 years) as well as duration of illness (<4 years versus ≥4 years). Glycaemic control was assessed based on glycosylated haemoglobin (HbA1c) level, where an HbA1c[19] value of <8.0% was considered to indicate "well-controlled" diabetes mellitus. These data were documented from routine or standard clinical practice in the management of all children and teenagers with T1DM.

Sample size

All 53 T1DM patients, between 6 to 18 years old receiving treatment at the Paediatric Endocrine Unit in Hospital Tunku Azizah Kuala Lumpur between April 29, 2019 to July 15, 2021 were invited to participate in the study.

A minimum sample size of 47 achieves 80% power to estimate the prevalence of psychosocial problems among

adolescents with T1DM assuming that the prevalence of this outcome is within (56% ± 5%)[20] with 95% confidence level. This sample size was adjusted for 15% non-response.

Statistical analysis

Data was analysed using IBM SPSS Statistics version 22.[21] Quantitative data with normal distributions were expressed as mean ± SD. The T-scores were categorised as normal or abnormal and expressed as frequencies, and percentages. Chi-square test and Fisher's exact test were used to make comparisons between the categorised T-scores and the demographic variables. For all tests, the level of significance was set at 0.05. Pearson's correlation and simple linear regression analysis were performed to evaluate the associations between global scale T scores with age, duration of illness and HbA1c.

Ethics

Approval from the Medical Research and Ethics Committee (MREC) in the National Institutes of Health Malaysia located in Selangor, Malaysia was obtained with reference number NMRR-18-2930-43850 (IIR).

RESULTS

Demographic characteristics

A total of 53 parents of T1DM patients who met the inclusion criteria were approached but only 46 agreed to participate in the study. Out of the 46 respondents, 4 dropped out and 2 had incomplete data and therefore were excluded. In the final sample, there were 40 respondents. The demographic characteristics of the patients are shown in Table 1. The mean age was 12.4 years (SD = 2.69), 52.5% were males, and the majority (75.0%) were Malays. Mean age of onset was 7.6 years (SD = 2.81) while the average duration of illness was 4.8 years (SD = 2.89). The majority (75%) of the patients had poor glycaemic control with a mean HbA1c of 9.4% (SD = 2.30). Nine were pre-pubertal (22.5%) while 31 were pubertal (77.5%).

CBCL scores

In the analysis, the borderline range and clinical range scores were grouped together as abnormal due to the small number in both groups. As shown in Table 2, 32.5% of the respondents have abnormal total scores, 35% had some form of internalizing problems and 17.5% had some form of externalizing problems.

CBCL scores by subgroups

Comparisons of CBCL subscales were made between two age groups of: ≤12 years and >12 years. The differences were tested using chi-square test and Fisher's exact test. The results are presented in Tables 3-5. More older children had abnormal CBCL scores than younger children. There was a significant difference in parent-reported internalizing problems ($p = 0.004$) between the two age groups, especially in the anxious/depressed syndrome subscale ($p = 0.001$) (Table 6). In the other parent-reported problem areas, overall, a higher proportion of the older children had problems. However, the differences were not statistically significant.

Aside from this, the three main global scores were compared by gender, glycaemic control, duration of illness and pubertal status. The results are shown in Tables 3-5. In proportion, females had more parent-reported problems in all the 3 global scores. Similarly, those with poor glycaemic control, longer duration of illness (only for internalizing problems, not global score) and those who have achieved puberty, showed higher scores in the global scores. However, the differences were not statistically significant.

Similarly, Pearson's correlation also showed a significant correlation between age and total scores and internalizing scores (Table 7).

Table 1. Demographic characteristics and clinical characteristics of patients

	n = 40
Age (yr)*	12.4 ± 2.69
Gender	
Male	21 (52.5%)
Female	19 (47.5%)
Race	
Malay	30 (75.0%)
Chinese	5 (12.5%)
Indian	5 (12.5%)
Pubertal status	
Pre-pubertal	9 (22.5%)
Pubertal	31 (77.5%)
Onset of diagnosis (yr)*	7.6 ± 2.81
Disease duration (yr)*	4.8 ± 2.89
HbA1c (%)*	9.4 ± 2.30
Glycaemic control	
Well-controlled	10 (25%)
Poor-controlled	30 (75%)

*values are presented in mean ± standard deviation

Table 2. Types of problems comparing those with normal and abnormal CBCL scores

	n = 40	
	Normal CBCL score	**Abnormal CBCL score**
Total problems	**27 (67.5%)**	**13 (32.5%)**
Internalizing problems	**26 (65.0%)**	**14 (35.0%)**
Anxious/depressed	32 (80.0%)	8 (20.0%)
Withdrawn/depressed	32 (80.0%)	8 (20.0%)
Somatic complaints	28 (70.0%)	12 (30.0%)
Externalizing problems	**33 (82.5%)**	**7 (17.5%)**
Rule-breaking behaviour	36 (90.0%)	4 (10.0%)
Aggressive behaviour	36 (90.0%)	4 (10.0%)
Others		
Attention problems	33 (82.5%)	7 (17.5%)
Social problems	34 (85.0%)	6 (15.0%)
Thought problems	37 (92.5%)	3 (7.5%)

Table 3. Comparison of CBCL scores according to age, gender, glycaemic control, duration of illness and pubertal status *(Total problems)*

	Total n	Abnormal, n (%)	Normal, n (%)	*p*
Age (years)				0.217[a]
≤12	21	5 (23.8%)	16 (76.2%)	
>12	19	8 (42.1%)	11 (57.9%)	
Sex				0.056[a]
Male	21	4 (19.0%)	17 (81.0%)	
Female	19	9 (47.4%)	10 (52.6%)	
Glycaemic control				1.000[b]
Poor-controlled	30	10 (33.3%)	20 (66.7%)	
Well-controlled	10	3 (30.0%)	7 (70.0%)	
Duration of illness (years)				0.581[a]
<4	16	6 (37.5%)	10 (62.5%)	
≥4	24	7 (29.2%)	17 (70.8%)	
Pubertal status				0.690[b]
Pre-pubertal	9	2 (22.2%)	7 (77.8%)	
Pubertal	31	11 (35.5%)	20 (64.5%)	

[a] Chi square test; [b] Fisher's exact test

Table 4. Comparison of CBCL scores according to age, gender, glycaemic control, duration of illness and pubertal status *(Internalizing problems)*

	Total n	Abnormal; n (%)	Normal; n (%)	*p*
Age (years)				0.004[a]
≤12	21	3 (14.3%)	18 (85.7%)	
>12	19	11 (57.9%)	8 (42.1%)	
Sex				0.119[a]
Male	21	5 (23.8%)	16 (76.2%)	
Female	19	9 (47.4%)	10 (52.6%)	
Glycaemic control				0.718[b]
Poor-controlled	30	10 (33.3%)	20 (66.7%)	
Well-controlled	10	4 (40.0%)	6 (60.0%)	
Duration of illness (years)				0.685[a]
<4	16	5 (31.3%)	11 (68.7%)	
≥4	24	9 (37.5%)	15 (62.5%)	
Pubertal status				0.124[b]
Pre-pubertal	9	1 (11.1%)	8 (88.9%)	
Pubertal	31	13 (41.9%)	18 (58.1%)	

[a] Chi square test; [b] Fisher's exact test

DISCUSSION

Young people with diabetes appear to have a greater incidence of depression, anxiety, psychological distress, and eating disorders compared to their healthy peers.[22-24] In our study, 35% of the respondents had parent-reported internalizing problems and 17.5% had parent-reported externalizing problems. The result of our study is similar to another study which reported that internalizing problems were more common compared with externalizing problems.[25]

By age group, those >12 years had statistically significantly more internalizing problems compared to the younger ones, especially in the anxious/depressed and withdrawn/depressed syndrome scales. These problems may be associated with (1) a decline in self-esteem during adolescence; (2) peer pressure when they compare their restrictive lifestyle with their peers; (3) lack of social acceptance including school bullying; (4) changes in school environment from primary school to secondary school.

T1DM patients are at risk of developing psychosocial complications, such as anxiety and depression. However, they often do not have proper psychiatric evaluation and support. This may adversely affect their management and disease control. A previous study showed that those who had higher CBCL scores had poorer glycaemic control.[26] A meta-analysis of 24 studies also showed that depression in diabetic patients was significantly associated with hyperglycaemia.[27] The poor-control group had higher scores for somatic complaints and withdrawal.[28]

Guidelines have previously recommended that practitioners managing children and adolescents with T1DM should have resources made available to them including professionals with expertise in the mental and behavioural health of children and adolescents such as psychologists, social workers, and psychiatrists.[29] These professionals should work within an interdisciplinary diabetes health care team. In our local setting, there is a need for the development of preventive and management strategies to promote behavioural and emotional well-being among young people with diabetes. This includes increasing awareness among healthcare workers as well as increasing the resources of social welfare, psychological and counselling services which are limited in our local setting.

In contrast to previous studies, those with poor glycaemic control in our study did not show a statistically significant

Table 5. Comparison of CBCL scores according to age, gender, glycaemic control, duration of illness and pubertal status *(Externalizing problems)*

	Total n	Abnormal, n (%)	Normal, n (%)	*p*
Age (years)				0.226[b]
≤12	21	2 (9.5%)	19 (90.5%)	
>12	19	5 (26.3%)	14 (73.7%)	
Sex				0.226[b]
Male	21	2 (9.5%)	19 (90.5%)	
Female	19	5 (26.3%)	14 (73.7%)	
Glycaemic control				0.656[b]
Poor-controlled	30	6 (20.0%)	24 (80.0%)	
Well-controlled	10	1 (10.0%)	9 (90.0%)	
Duration of illness (years)				1.000[b]
<4	16	3 (18.8%)	13 (81.2%)	
≥4	24	4 (16.7%)	20 (83.3%)	
Pubertal status				1.000[b]
Pre-pubertal	9	1 (11.1%)	8 (88.9%)	
Pubertal	31	6 (19.4%)	25 (80.6%)	

[b] Fisher's exact test

Table 6. Comparison of CBCL subscores between age ≤12 years and >12 years

	≤12 years, n = 21 (%)		>12 years, n = 19 (%)		*P*
	Normal	Abnormal	Normal	Abnormal	
Total problems	16 (76.2%)	5 (23.8%)	11 (57.9%)	8 (42.1%)	0.217[a]
Internalizing problems	18 (85.7%)	3 (14.3%)	8 (42.1%)	11 (57.9%)	0.004[a]
Anxious/depressed	21 (100.0%)	0 (0.0%)	10 (52.6%)	9 (47.4%)	0.001[b]
Withdrawn/depressed	19 (90.5%)	2 (9.5%)	12 (63.2%)	7 (36.8%)	0.060[b]
Somatic complaints	17 (81.0%)	4 (19.0%)	11 (57.9%)	8 (42.1%)	0.112[a]
Externalizing problems	19 (90.5%)	2 (9.5%)	14 (73.7%)	5 (26.3%)	0.226[b]
Rule-breaking behaviour	20 (95.2%)	1 (4.8%)	19 (100.0%)	0 (0.0%)	1.000[b]
Aggressive behaviour	20 (95.2%)	1 (4.8%)	16 (84.2%)	3 (15.8%)	0.331[b]
Others					
Attention problems	19 (90.5%)	2 (9.5%)	16 (84.2%)	3 (15.8%)	0.654[b]
Social problems	19 (90.5%)	2 (9.5%)	15 (78.9%)	4 (21.1%)	0.398[b]
Thought problems	21 (100.0%)	0 (0.0%)	16 (84.2%)	3 (15.8%)	0.098[b]

[a] Chi square test; [b] Fisher's exact test

Table 7. Correlation between Global scale T scores with age, duration of illness and HbA1c

		R	*P*
Age (n = 40)	*Internalizing*	0.405	0.005
	Externalizing	0.216	0.090
	Total	0.321	0.022
Duration of illness (n = 40)	*Internalizing*	0.083	0.306
	Externalizing	0.076	0.320
	Total	0.005	0.487
HbA1c (n = 40)	*Internalizing*	0.074	0.325
	Externalizing	0.226	0.081
	Total	0.154	0.171

R: Pearson's correlation coefficient

difference in CBCL scores.[10-14,30] In a similar study conducted in China, compared with a control group, the well-controlled T1DM patients had higher scores for withdrawal, anxiety/depression, and internalizing problems while the poorly-controlled T1DM patients had higher scores for withdrawal, somatic complaints, anxiety/depression, delinquent behaviours, aggressive behaviours, externalizing and internalizing problem.[7] The majority (75%) of the patients in our study had poor glycaemic control, and this factor may dilute any potential association between poor glycaemic control and behavioural and emotional issues. This suggests that there may be factors negatively impacting their overall glycaemic control such as a lack of awareness about the disease and its proper management among others. Determining non-compliance to treatment and other factors resulting to poor glycaemic control in T1DM, and emphasizing the importance of good control through re-education and counselling need to be instituted and practised.

Our study also showed that gender had no association with CBCL scores, which is similar with the findings from previous studies from Asian countries.[26,28] However, a greater proportion of female patients with T1DM do tend to have abnormal scores in total as well as internalizing and externalizing problems, with total problems nearly reaching significant levels. This suggests that female patients may be struggling with psychological issues more than expected. Puberty is associated with poorer glycaemic control due to its association with a decrease in insulin sensitivity.[8] However, similar to glycaemic control and gender subgroups, T1DM patients during puberty did not show significantly higher CBCL scores.

In contrast to a previous study which showed that duration of illness had a significant association with CBCL scores[26], this association was not observed in our study. One reason to account for this could be differences in disease duration classification used (<4 years or <1 year) in different studies. In our study, there are only 5 patients with ≤1-year duration from the onset among the total of 40 patients. However, even after analysing the cut-off point of 1 year, the variable did not demonstrate an association with

CBCL scores. A larger sample size may help establish the association better as our study was not adequately powered to establish the associations between these possible factors and behavioural or emotional problems. A multicentre study may be necessary to provide more information on its association.

The education level of caregivers is an important contributing factor in the management of these patients. Previous studies found an association between maternal education levels and internalization problems.[25] However, this was not evaluated in this study as this data was not collected. It is important to include maternal education in future studies as it may play a role in the development of behavioural and emotional problems in children and adolescents with T1DM.[25]

Limitations

This assessment is based on parents' perspective and may not truly reflect what the children are going through. As in all self-administered surveys, whether the respondents answered the questions honestly cannot be assessed, and thus responses are taken at face value. This study was conducted in only one hospital and the sample size was small. Our study was inadequately powered to investigate the associated factors which may be linked to behavioural and emotional problems. The small sample size is likely the greatest limitation of the study, especially when assessing association of specific factors within subsamples, making generalisability of the findings to the general population challenging. A multicentre study with a large sample size would be more informative. Further information, such as parental education level and socioeconomic status, were also not collected in this study.

CONCLUSION

This study showed that TIDM children and adolescents >12 years old are at higher risk of developing psychosocial difficulties such as anxiety and depression. It is important to recognise and screen for symptoms of psychosocial complications in this group of patients to make an early diagnosis and address them, as well as involve psychologyand mental health professionals as part of a multi-disciplinary team to improve the overall care of T1DM patients.

We recommend future studies in this field to further elucidate issues, and suggest a much larger multi-centre study to establish the association between factors which may be linked with adverse behaviours and psychological outcomes among children and adolescents with T1DM.

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Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
WLC: Formal analysis, Investigation, Data Curation, Writing – original draft preparation, Visualization; **ANI:** Conceptualization, Methodology, Validation, Resources, Writing – review and editing, Supervision, Project administration; **NKN:** Resources, Writing – review and editing; **LPG:** Writing – review and editing.

Conflict of Interest
The authors have no conflicts of interest to disclose.

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ORIGINAL ARTICLE

A Cross-sectional Study to Assess Beta-Cell Function in Individuals with Recently Diagnosed Young-Onset Type 2 Diabetes Mellitus and Its' Complications

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Abstract

Objective. The primary objective was to assess beta-cell function of recently-diagnosed young-onset type 2 diabetes mellitus (T2DM) individuals using basal and stimulated C-peptide levels. The secondary objective was to examine the association between C-peptide with metabolic factors and diabetes complications.

Methodology. A cross-sectional study was conducted for young-onset T2DM individuals aged 18-35 years with a disease duration of not more than 5 years. Plasma C-peptide was measured before and after intravenous glucagon injection. Demographic data, medical history and complications were obtained from medical records and clinical assessment. Continuous data were expressed as median and interquartile range (IQR). Categorical variables were described as frequency or percentage. Multivariable linear regression analysis was used to determine factors associated with C-peptide levels.

Results. 113 participants with young-onset T2DM with a median (IQR) age of 29.0 (9.5) years and 24 (36) months were included in this study. The median (IQR) basal and stimulated C-peptide was 619 (655) pmol/L and 1231 (1024) pmol/L. Adequate beta-cell function was present in 78-86% of the participants based on the basal and stimulated C-peptide levels. We found hypertension, obesity and diabetic kidney disease (DKD) to be independently associated with higher C–peptide levels. In contrast, females, smokers, those on insulin therapy and with longer duration of disease had lower C–peptide levels.

Conclusion. Most recently diagnosed young-onset T2DM have adequate beta-cell function. Elevated C-peptide levels associated with obesity, hypertension and diabetic kidney disease suggest insulin resistance as the key driving factor for complications.

Key words: type 2 diabetes mellitus, young-onset, beta-cell function, C-peptide, glucagon stimulation test

INTRODUCTION

Young-onset type 2 diabetes mellitus (T2DM) is defined as onset of T2DM before the age of 40 years in the absence of secondary causes.[1] Studies have shown an alarming increase in the prevalence of young-onset T2DM globally, more so in Asia in the last few decades.[2] The International Diabetes Federation (IDF) estimated an increase from 23 to 63 million young adults worldwide to have T2DM from 2000 to 2013 with the biggest increase being in Africa, Southeast Asia, and the Western Pacific region.[2] In an Asian study, approximately 20% of patients with type 2 diabetes were young-onset T2DM.[3] This subset of individuals has been associated with accelerated disease progression and premature complications.[2] There are also higher rates of insulin commencement and intensification of treatment regimen early in the disease compared to the usual onset.[1]

The pathophysiology of T2DM in the young has been thought to be similar to the usual onset with an interplay of beta-cell dysfunction, insulin resistance and obesity-related mechanisms.[2,4] There is limited data with regards

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to beta-cell function in the early stage of the disease in this population. Objective assessment of beta-cell function is important to assist us in understanding the disease mechanism and rate of progression, as well as to guide therapeutic options. This is a pilot study performed in Malaysia to assess beta-cell function of recently-diagnosed young-onset T2DM individuals using fasting and glucagon stimulated C-peptide levels. C-peptide is produced in equal amounts to insulin and is an objective marker of beta-cell function.[5] Glucagon stimulation test (GST) is an easily performed test to assess stimulated C-peptide with good sensitivity and reproducibility in clinical practice.[6] The secondary objective of this study was to examine the association among C-peptide levels with metabolic parameters and diabetes-related complications.

METHODOLOGY

Study design and participants

This is a cross-sectional study involving young-onset T2DM individuals seen in the diabetes clinics of two urban tertiary hospitals in Malaysia between September 2019 to December 2020. Individuals aged 18 to 35 years diagnosed with T2DM for not more than five years were recruited using the universal sampling method. This was a descriptive study, therefore no formal sample size was required. We excluded individuals with chronic kidney disease stage 2 and above (estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2); concurrent infection or inflammatory disease, recent diabetic ketoacidosis or hyperglycaemic hyperosmolar state in the last three months, positive diabetes autoantibodies, prior clinical diagnosis of other forms of diabetes (monogenic diabetes, type 1 diabetes, latent autoimmune diabetes of adult onset and secondary diabetes), fasting capillary blood glucose less than 4.0 mmol/L or more than 13.9 mmol/L on the day of testing, and those who were pregnant.[7] Written informed consent was obtained from all study participants prior to the commencement of the study. This study was approved by the Medical Research and Ethics Committee (MREC) of the Ministry of Health of Malaysia.

Study recruitment and procedures

Study participants were recruited during their routine clinical visits to the diabetes clinic. Individuals with unknown diabetes mellitus autoantibodies status were screened prior to enrolment. Those with positive diabetes autoantibodies were excluded from the study. This study involved only a single visit. Study participants were required to fast overnight for at least eight hours and to omit all insulin and oral antidiabetic agents on the morning of testing. On the day of testing, anthropometric measurements (weight, height, waist circumference) were taken using calibrated tools. Vital signs and capillary blood glucose pre-procedure were checked. GST was performed if fasting capillary glucose levels were between 4.0 to 13.9 mmol/L. Basal C-peptide and blood glucose levels were sampled prior to administration of 1 mg of intravenous glucagon. After 6 minutes, stimulated blood glucose and C-peptide samples were collected. Study participants were monitored for adverse effects for 15 minutes post testing. All tests were conducted by a single operator. Information regarding demography, disease history, co-morbidities, complications and treatment were gathered from medical records and clinical assessment.

Measures

The primary outcome of this study, beta-cell function, was measured using C-peptide levels (fasting and stimulated). Adequate beta-cell function was defined as either a basal C-peptide level of more than 250 pmol/L or a stimulated C-peptide level of more than 600 pmol/L, or both.[5]

Independent variables examined included current age, gender, ethnicity, smoking status, family history of T2DM, age of disease onset, disease duration, fasting glucose, HbA1c, insulin therapy, waist circumference, obesity, hypertension, dyslipidaemia, macrovascular complications, microvascular complications, retinopathy, nephropathy, and neuropathy. Waist circumference was measured midway between the iliac crest and the lowermost margin of the ribs at the end of a normal respiratory expiration.[8] Obesity was defined based on a body mass index (BMI) cut-off of ≥27.5 kg/m^2.[9] Abdominal obesity was defined as a waist circumference of ≥90 cm in males or ≥80 cm in females.[8] Glycated haemoglobin (HbA1c) performed in the last three months was used as a measure of glycaemic control. Hypertension was defined as the persistent elevation of systolic blood pressure of 140 mmHg or greater and/or a diastolic blood pressure of 90 mmHg or greater.[10] Dyslipidemia in type 2 diabetes was diagnosed based on low-density lipoprotein (LDL) cholesterol levels of 2.60 mmol/l or greater, high-density lipoprotein (HDL) cholesterol levels of 1.02 mmol/l or less and triglyceride levels of 1.7 mmol/l or greater.[11]

Macrovascular complication was defined as an established history of ischemic heart disease, stroke or peripheral vascular disease based on medical records. Microvascular complication was defined as the presence of one or more of the following complications: retinopathy, nephropathy or peripheral neuropathy. Retinopathy was assessed based on slit lamp examination records performed in the last 12 months by credentialed personnel at the ophthalmology clinic of respective tertiary hospitals. Participants with no recent assessment were referred for evaluation as per routine protocol. Both ophthalmology clinics used the Early Treatment for Diabetic Retinopathy Study (ETDRS) classification to diagnose and classify diabetic retinopathy. Diabetic kidney disease (DKD) was assessed based on two or more urinary spot quantification of proteinuria or urine albumin creatinine ratio (ACR) performed in the last 12 months. Microalbuminuria was defined as a urine ACR of 3-30 mg/mmol whereas overt nephropathy was defined as a urine ACR of >30 mg/mmol.[12] Subjects with chronic

kidney disease stage 2 and above (estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2) were excluded from this study, as majority of C-peptide is metabolised by the kidney and may result in inaccurate results.[5] Peripheral neuropathy was diagnosed based on diminished sensation to standardised monofilament examination or reduced vibration on graduated tuning fork testing. Successful weaning of initial insulin therapy was defined as the ability to discontinue insulin in those initiated on insulin within 6 months of diagnosis.

C-peptide analysis was performed in a centralised laboratory using IMMULITE-2000 C-peptide (Siemens), a solid phase, two-site chemiluminescence immunometric assay. The co-efficient of variation (CV) of this test is less than 5% based on the designated laboratory internal quality control (IQC) performance analysis. This is in keeping with target of desirable biological variation.[13] Study participants were screened for diabetes autoantibodies including anti-islet cell antibodies (ICA), anti-glutamic acid (GAD) antibodies and anti-islet tyrosine phosphatase (IA2) antibodies using commercially available ELISA kits (Medipan GmbH, Germany). Fasting blood glucose level was determined by hexokinase method. Urinary albumin and creatinine were measured by immunoturbidimetric and enzymatic methods respectively. Lastly, HbA1c was analysed using high performance liquid chromatography (HLPC).

Statistical analysis

All statistical analysis was performed with Statistical Package for Social Science (SPSS) Version 22.0.[14] Most continuous data was found to be not normally distributed, therefore expressed as median and interquartile range (IQR). Categorical variables were described as frequency or percentage. Bivariate analysis using Mann Whitney U and Kruskal Wallis test were used to examine association between categorical data. Spearman's rank correlation coefficient was used to assess relationship between continuous data. We proceeded with linear regression analysis using variables with $p<0.25$ from bivariate analysis and clinically important outcome variables based on biological plausibility. Stepwise linear regression with forward selection was performed. The final model was tested for autocorrelation (Durbin-Watson test), multicollinearity and homoscedasticity. The significance level for all steps of analyses was $p<0.05$.

RESULTS

A total of 151 patients were screened to be recruited in this study. Twenty-eight patients were excluded due to positive autoantibodies (n = 20), CKD stage 3 and above (n = 2), pregnancy (n = 1), recent diabetic ketoacidosis (n = 2), and hyperglycemia on day of testing (n = 3). Six patients declined participation and the remainder (n = 4) were not contactable. A final sample size of 113 participants participated in this study.

Patient characteristics (Table 1)

The median (IQR) age and disease duration of study population was 29 (9.5) years and 24 (36) months respectively. There was a female preponderance (n = 70, 61.9%) with the predominant ethnicity being Malay (n = 80, 70.8 %). More than two-thirds (n = 84, 74.3%) of the study population was obese. Approximately 90% of the subjects had abdominal obesity: 88.4% of the males (n = 38) and 91% of the females (n = 64). Almost all had concomitant dyslipidaemia (n = 104, 92.0%) while less than one-third had hypertension (n = 34, 30.1%). Majority had a family history of T2DM involving at least one first-degree relative (n = 94, 83.2%). There were 14.2% (n = 16) active smokers in the population studied. The average glycaemic control of the population studied was poor, with a median (IQR) HbA1c of 8.5% (4.1). More than half of the study population (n = 66, 58.4%) was currently on insulin therapy. It was found that 75.3% (n = 55) of all patients with history of insulin use were initiated on insulin therapy early (within six months from diagnosis). However, only 15.7% (n = 8) of those started on early insulinization were successfully weaned off. There were no documented macrovascular complications; however, microvascular complications were present in 38.1% (n = 43) of the cohort. DKD was the most common microvascular complication (34.5%, n = 39), mostly in the form of microalbuminuria. Retinopathy and peripheral neuropathy were observed in less than 10% of the cohort.

Glucagon stimulation test results (Table 2)

The C-peptide levels had a median (IQR) basal value of 619 (655) pmol/L and 1231 (1024) pmol/L with stimulation. There was a strong positive correlation between fasting and stimulated C-peptide levels, rs = 0.92, $p<0.001$ (data not shown in Table 2). Majority of the study participants had adequate beta-cell function based on the basal and stimulated C-peptide levels at 86% and 78% respectively. Within the subgroup of participants on insulin as current therapy, there was still a high proportion registering adequate beta-cell function at 77% and 70% respectively based on the basal and stimulated C-peptide levels. Transient and self-limiting nausea was reported in 8.8% (n = 10) participants immediately after the procedure.

Factors associated with basal and stimulated C-peptide levels

Table 3 shows the bivariate analysis between basal and stimulated C-peptide levels with baseline clinical characteristics, metabolic parameters and diabetes-related complications. Both basal and stimulated C-peptide were significantly associated with gender, disease duration, age of disease onset, HbA1c, insulin therapy, obesity, waist circumference and hypertension ($p<0.05$). In addition, stimulated C-peptide was also significantly associated with current age, smoking and dyslipidaemia ($p<0.05$). There was no significant association between basal and stimulated C-peptide with diabetes related complications.

We proceeded with linear regression analysis to determine factors independently associated C-peptide levels (Tables 4 and 5). We included 11 variables with $p<0.25$ from the bivariable analysis and clinically important outcome variables studied into the preliminary main effect model (current age, gender, smoking, age of disease onset, disease duration, HbA1c, obesity, insulin therapy, dyslipidaemia, hypertension and DKD). We found hypertension, obesity and DKD to be independently associated with higher C–peptide levels. In contrast, females, smokers, those on insulin therapy and those with longer disease duration had lower C–peptide levels.

DISCUSSION

We found our young-onset T2DM population to be predominantly female (62%). Subjects also had a strong family history of diabetes. Almost three-quarters of them were obese by BMI criteria. More than 90% had abdominal obesity associated with dyslipidaemia while almost one-third had hypertension. This high prevalence of metabolic syndrome with multiple cardiovascular risk factors despite a young age and short disease duration is similar to the clinical characteristics reported in literature.[1,3,15] The median HbA1c of 8.5% (69 mmol/mol) in our study cohort indicated a relatively poorer glycaemic control compared to the median HbA1c of 7.9% (63 mmol/mol) for the general diabetic population in the country.[16] Our findings concur

Table 1. Patient demography, metabolic parameters, disease history and complications

Variables			Median (IQR)[a]	n (%)
Demography	Age (years)		29.0 (9.5)	
	Gender	Male		43 (38.1)
		Female		70 (61.9)
	Ethnicity	Malay		80 (70.8)
		Chinese		15 (13.3)
		Indian		18 (15.9)
Metabolic parameters	Obesity	BMI <27.5 kg/m^2		29 (25.7)
		BMI ≥27.5 kg/m^2		84 (74.3)
	Abdominal Obesity	Male (WC ≥90 cm)		38 (88.4)
		Female (WC ≥80 cm)		64 (91.4)
	Hypertension			34 (30.1)
	Dyslipidaemia			104 (92.0)
Medical history	Diabetes duration (months)		24 (36)	
	HbA1c (%)		8.5 (4.1)	
	Family history of diabetes			94 (83.2)
	Smoking			16 (14.2)
Diabetes treatment history	Current insulin therapy			66 (58.4)
	Time to insulin initiation	≤6 months from diagnosis		55 (75.3)
		>6 months from diagnosis		18 (24.7)
	Successful weaning of initial insulin[b]			8 (15.7)
Complications	Microvascular			43 (38.1)
	Retinopathy			8 (7.1)
		Non-proliferative[c]		4 (50.0)
		Proiferative[c]		4 (50.0)
	Diabetic kidney disease			39 (34.5)
		Microalbuminuria[d]		29 (74.4)
		Overt nephropathy[d]		10 (25.6)
	Peripheral neuropathy			9 (8.0)

IQR, interquartile range; BMI, body mass index; WC, waist circumference.
[a] not normally distributed data expressed as median (IQR)
[b] within insulin initiation at ≤6 months from diagnosis subgroup
[c] within retinopathy subgroup
[d] within diabetic kidney disease subgroup

Table 2. Glucagon stimulation test results

Variables		Median (IQR) / n (%)
Glucose (mmol/L), median (IQR)	Fasting glucose	7.5 (3.3)
	Stimulated glucose	8.3 (3.7)
C-peptide (pmol/L), median (IQR)	Basal C-peptide	619.0 (655.0)
	Glucagon stimulated C-peptide	1231.0 (1024.0)
Beta cell function, n (%)	Adequate basal beta cell function[a]	97.0 (85.8)
	Adequate stimulated beta cell function[b]	88.0 (77.9)
	For participants on insulin as current therapy:	
	i. Adequate basal beta cell function[a]	51.0 (77.3)
	ii. Adequate stimulated beta cell function[b]	46.0 (69.7)

Results expressed in median (IQR) for not normally distributed variables and percentage for categorical variables.
[a] defined as C-peptide >250 pmol/L, [b] defined as C-peptide >600 pmol/L.[9]

with several studies done in the Asian region showing poorer glycaemic control in young-onset T2DM compared to the usual onset T2DM.[3,17]

The high percentage of insulin use within six months of diagnosis coupled with a low rate of subsequent weaning of insulin therapy in this study indicate a delayed diagnosis with high prevalence of severe hyperglycaemia at presentation requiring insulin, followed by difficulties in restoring euglycemia despite intensive use of insulin. This is consistent with the high percentage of undiagnosed diabetes among the young age group (18 to 39 years) which is at 77%

Table 3. Bivariate analysis between c-peptide and clinical features, metabolic parameters and diabetes complications

Variables		Basal C-peptide			Stimulated C-peptide		
		Median (IQR)[a]	r_s[b]	*p*	Median (IQR)[a]	r_s[b]	*p*
Current age			0.164	0.083		**0.259**	**0.006**
Gender	Male	857.0 (709.0)		**0.002**	1705.0 (1268.0)		**0.001**
	Female	541.0 (500.0)			1021.0 (980.3)		
Ethnicity	Malay	635.5 (658.5)		0.499	1247.5 (1008.3)		0.354
	Chinese	559.0 (469.0)			751.0 (1264.0)		
	Indian	639.0 (536.5)			1276.0 (902.0)		
Smoking	Yes	852.0 (625.5)		0.177	1673.5 (851.75)		**0.034**
	No	583.0 (637.0)			1086.0 (1071.5)		
Family history	Yes	625.5 (618.5)		0.693	1213.0 (952.0)		0.785
	No	543.0 (964.0)			1241.0 (1612.0)		
Age of disease onset			0.219	**0.020**		0.301	**0.001**
Disease duration			0.238	**0.011**		0.227	**0.015**
Fasting glucose			0.069	0.469		0.074	0.434
HbA1c			0.286	**0.002**		0.319	**0.001**
Insulin therapy	Yes	460.0 (382.0)		**<0.001**	917.0 (884.8)		**<0.001**
	No	910.0 (560.0)			1629.0 (954.0)		
Obesity[c]	Yes	766.5 (604.8)		**<0.001**	1493.0 (1108.8)		**<0.001**
	No	348.0 (322.5)			761.0 (553.0)		
Waist circumference			0.417	**<0.001**		0.404	**<0.001**
Dyslipidaemia	Yes	635.5 (681.5)		0.160	1259.5 (1036.0)		0.034
	No	450.0 (239.5)			970.0 (715.0)		
Hypertension	Yes	897.0 (870.3)		0.001	1622.0 (1085.5)		0.001
	No	546.0 (485.0)			1039.0 (1083.0)		
Microvascular	Yes	672.0 (688.0)		0.707	1347.0 (1434.0)		0.896
	No	594.5 (578.5)			1095.0 (975.3)		
Retinopathy	Yes	708.0 (1004.8)		0.392	1251.0 (1056.8)		0.771
	No	606.0 (645.0)			1231.0 (1038.0)		
Nephropathy	Yes	722.0 (685.0)		0.474	1413.0 (1373.0)		0.411
	No	581.0 (644.8)			1089.0 (1015.3)		
Neuropathy	Yes	424.0 (710.0)		0.321	1089.0 (1332.0)		0.284
	No	625.5 (641.5)			1236.0 (1004.8)		

[a] Mann-Whitney U test used for non-parametric binomial data and Kruskal Wallis for data with >2 categories
[b] Spearman's rank correlation coefficient (rs) used for non-parametric data
[c] Obesity defined as BMI ≥27.5 kg

Table 4. Factors independently associated with basal c-peptide levels from multivariable linear regression

Variables	Coefficient	95% Confidence Interval		*p*
		Lower limit	Upper limit	
Insulin therapy	-392.8	-550.1	-235.4	**<0.001**
Hypertension	192.0	22.6	361.5	**0.027**
Obesity	252.6	78.9	426.3	**0.005**
Female	-266.8	-434.9	-99.6	**0.002**
Nephropathy	186.0	27.2	344.9	**0.022**
Smoker	-264.7	-501.5	-28.0	**0.029**

Stepwise multiple linear regression was applied. The Durbin-Watson statistic, d=1.945 was between 1.5 and 2.5 and therefore data was not autocorrelated. Multicollinearity and homoscedasticity were checked and not found. A significant regression equation was found [F (6,104) = 13.076, $p<0.001$], with R^2 of 0.43.

Table 5. Factors independently associated with stimulated c-peptide levels from multivariable linear regression

Variables	Coefficient	95% Confidence Interval		*p*
		Lower limit	Upper limit	
Insulin therapy	-393.4	-689.6	-97.3	**0.010**
Hypertension	533.5	201.6	865.4	**0.002**
Obesity	382.4	49.2	715.8	**0.025**
Female	-355.1	-644.1	-66.1	**0.017**
Duration of Disease	-7.2	-14.4	-0.2	**0.044**

Stepwise multiple linear regression was applied. The Durbin-Watson statistic, d=1.907 was between 1.5 and 2.5 and therefore data was not autocorrelated. Multicollinearity and homoscedasticity were checked and not found. A significant regression equation was found [F (5,105) = 11.879, $p<0.001$], with R^2 of 0.3.

according to the National Health and Morbidity Survey in 2019.[18] In the Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) cohort, only 13% were initiated on insulin at diagnosis and nearly all were weaned off insulin during the run-in period of two to six months using metformin titration and diabetes education.[17] In the Asian region, Pan et al., showed that only 18% of newly-diagnosed young-onset diabetes patients used insulin during the first year of disease, despite approximately 10% of the study population having classical type 1 diabetes.[17]

There was a high prevalence of microvascular complication at 38% in our study cohort despite only a median disease duration of two years. There was a striking predominance of DKD among the microvascular complications, with majority having microalbuminuria. Literature shows the prevalence of microalbuminuria is higher among individuals with young onset T2DM at diagnosis and tends to occur after a shorter duration of disease.[2] The TODAY cohort showed a 6% prevalence of microalbuminuria at baseline whichit increased to 17% after four years.[19] The Pima Indian youth with T2DM (aged <20 years) had a much higher prevalence of microalbuminuria at diagnosis at 22% and was projected to reach 60% before the age of 30.[2] Kim et al., also reported a high prevalence of microalbuminuria at 34.3%, nearly 2-fold higher in those with newly-diagnosed young-onset T2DM compared to those diagnosed after the age of 40 years.[15] This indicates an increased risk of early-onset DKD with rapid progression among the young-onset T2DM individuals compared to usual onset, with possible ethnic variation.

C-peptide level was chosen as the measure of beta-cell function because it is a reliable indicator of insulin secretion. It has a longer half-life and is less affected by first-pass hepatic metabolism when compared to insulin. It also can be used to assess endogenous insulin production including patients on insulin treatment.[5,6] The median C-peptide in our cohort was 619 pmol/L (basal) and 1231 pmol/L (glucagon stimulated). Based on the recommended thresholds of 250 pmol/L (basal) and 600 pmol/L (stimulated) C-peptide levels, majority (78-86%) of our patients have adequate beta-cell function.[5] These results are comparable to other cohorts reported in the region.[15,17]

Kim et al., reported a median basal C-peptide of 788 pmol/L and 2-hour postprandial C-peptide of 2023 pmol/L in their newly-diagnosed young-onset T2DM.[15] Pan et al., reported a mean basal C-peptide of 700 pmol/l with an almost identical percentage (80-83%) of adequate beta-cell function which was assessed using basal and glucagon stimulation C-peptide testing in their newly diagnosed young-onset diabetes individuals consisting mainly of T2DM.[17] This is in contrast to some other studies that reported significant beta cell dysfunction in newly-diagnosed young-onset T2DM.[20,21] Even within the subgroup of insulin users, there was a high proportion of at least 70% having adequate beta-cell function. Pan et al., showed a lower percentage, with approximately 50% of their young newly-diagnosed DM on insulin with adequate basal and stimulated beta-cell function using similar C-peptide cut off levels.[17] However, their study population included those with T1DM.[17]

We postulated that the high fasting and stimulated C-peptide level shown in our study may be a surrogate marker of insulin resistance in this population.[6,22] Therefore, we proceeded with post-hoc analysis to calculate modified HOMA-IR levels to assess insulin resistance in this cohort using basal C-peptide levels.[23] We found a mean ± standard deviation (SD) modified HOMA-IR of 3.48 ± 1.36. The modified HOMA-IR values showed a strong positive correlation with basal C-peptide levels, rs = 0.90, p<0.001. Based on previous studies done in the Asian region, a HOMA-IR value of more than 2.5 has been used to define insulin resistance.[24,25] Using this cut off, 78% of our study population were found to be insulin resistant. This further supports our hypothesis that our recently-diagnosed young-onset T2DM population was predominantly insulin resistant as opposed to having inadequate beta-cell function.

Linear regression analysis in this study revealed females to have lower C-peptide levels than males. This may indicate a possible difference in beta-cell function between genders among the young-onset T2DM. Despite the known preponderance of females in the young-onset T2DM, there has been no conclusive evidence to suggest difference in beta-cell function between genders to date.[2]

Obesity and hypertension were independently associated with higher basal and stimulated C-peptide levels. This is presumably because an elevated C-peptide is known to be a surrogate marker of insulin resistance in individuals with the metabolic syndrome phenotype.[6]

As expected, patients on insulin therapy were found to have significantly lower basal and stimulated C-peptide levels. There has been concern in literature about suppression of endogenous insulin and C-peptide secretion in those on exogenous insulin therapy when beta-cell function is assessed. However, it has been proven that acute normalization of glucose concentration or hypoglycaemia is responsible for suppression of C-peptide levels during testing instead of the direct effect of the exogenous insulin.[6,26]

Gjessing et al., has shown that contrary to the hypothesis of beta-cell function attenuation due to glucotoxicity, fasting hyperglycaemia in fact potentiates stimulated C-peptide levels during mixed-meal tolerance testing or glucagon stimulation testing in T2DM.[27] In this study, we ensured that the blood glucose of study participants was between 4.0 to 13.9 mmol/L at the time of testing to minimize the possible effect of extreme glucose concentrations on C-peptide values.[7]

We also found active smokers to have lower basal C-peptide levels than non-smokers. Although there are epidemiological studies confirming smoking as a risk factor for the development of T2DM, the effect of smoking on beta-cell function and insulin resistance is not conclusive.[28–31]

DKD was found to be independently associated with elevated basal C-peptide levels in this study. In our study cohort, there was a high prevalence of DKD early in the disease despite majority of them having adequate beta-cell function. Older studies done in the usual-onset T2DM with longer disease duration showed an opposite relationship with lower C-peptide levels associated with DKD.[7,22]

A recent large data-driven cluster analysis by Ahlqvist et al.,in Scandinavia identified five novel subgroups in their T2DM population. These five subgroups were classified as severe autoimmune diabetes (SAID), severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD) and mild age-related diabetes (MARD).[32] The SIRD subgroup characterized by obesity and insulin resistance had a higher risk of DKD independent of their glycaemic control compared to the other subgroups.[32] Zou et al., confirmed these findings in the Chinese and United States populations.[33] However, Anjana et al., identified an additional subgroup, Combined Insulin Resistant and Deficient Diabetes (CIRDD), unique to the Asian Indian population. This subgroup was younger in onset, had combined insulin deficiency and resistance with poor glycaemic control and increased hazards of kidney disease and retinopathy.[34] A similar analysis in our young-onset T2DM population will be beneficial to understand the subgroups within and their characteristics, including risk of complications.

Limitations

This study had a few limitations. Although this was a descriptive study not requiring a formal sample size, the modest sample size may have underscored the power in detecting the association between C-peptide with metabolic parameters and diabetes-related complications. Therefore, less emphasis can be placed on significance testing of results in this study. The cross-sectional study design was not able to establish causal relationship between measured variables and outcomes.

Our study population was comprised of patients attending diabetes clinics in two urban tertiary hospitals and was not fully representative of the general population of young-onset T2DM in the country. Despite attempts to exclude those with monogenic diabetes based on prior clinical diagnosis, the lack of genetic testing is a limiting factor.

Lastly, the slit lamp examination not being done by designated ophthalmologists may have lead to operator bias in the results of retinopathy screening. However, it was verified that the standard operating procedure of both of the ophthalmology clinics required their personnel to be credentialed and privileged to perform the test and used the Early Treatment for Diabetic Retinopathy Study (ETDRS) classification to diagnose and classify diabetic retinopathy.

This is the first study assessing beta-cell function in recently diagnosed, exclusively young-onset T2DM in the country and one of the few in Asia. Various measures to minimize the effects of confounding factors on C-peptide measurement were undertaken including stringent eligibility criteria, exclusion of extreme blood glucose levels and omission of exogenous insulin on the day of testing to improve the accuracy of C-peptide testing.

CONCLUSION

Majority of the participants who have young-onset diabetes have adequate basal and stimulated pancreatic beta-cell function. There was a high rate of metabolic syndrome in this study cohort associated with early-onset DKD. The glycaemic control was poor despite high rates of insulin use. Elevated C-peptide levels associated with obesity, hypertension and DKD suggest that insulin resistance rather than beta-cell dysfunction is the key driving factor of complications. Our study paves the direction for future prospective studies involving a larger cohort of individuals with direct measurement of insulin sensitivity to confirm our findings. Treatment strategies in this population should be tailored, focusing on early diagnosis before development of severe hyperglycaemia with a combination of intensive lifestyle modification and pharmacotherapy to address obesity, insulin resistance and avoiding overtreatment with insulin. These individuals should be screened for microvascular complications especially DKD upon diagnosis and monitored closely thereafter to prevent premature morbidity and mortality.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
SN: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing - original draft preparation, Visualization, Project administration, Funding acquisition; **SR:** Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – review and editing, Visualization; **MBLB:** Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – review and editing, Visualization; **NSCR:** Conceptualization, Methodology, Validation, Investigation, Resources, Visualization; **ST:** Conceptualization, Methodology, Validation, Investigation, Resources, Visualization; **NA:** Conceptualization, Methodology, Validation, Investigation, Resources, Visualization; **YMC:** Conceptualization, Methodology, Validation, Investigation, Resources, Visualization; **SHF:** Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – review and editing, Supervision, Project administration, Funding acquisition.

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ORIGINAL ARTICLE

Severity and Factors Associated with Depressive Symptoms Among Type 2 Diabetic Patients in Vietnam

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Abstract

Background. Diabetes and psychiatric disorders often co-occur. The prevalence of depression in a person with diabetes is two times higher than that of the general population. During the last decade, the prevalence of diabetes in Vietnam has nearly doubled. However, there is little data regarding depressive symptoms among people with diabetes. Therefore, this study aims to explore the level of depressive symptoms and its associated factors among patients with type 2 diabetes mellitus in Hanoi, Vietnam.

Methodology. A cross-sectional study was conducted among 519 patients diagnosed with type 2 diabetes at the Agricultural General Hospital, one of the largest primary care hospitals for diabetes in Hanoi, Vietnam. Patient Health Questionnaire-9 (PHQ-9) was used to assess the severity of depressive symptoms. Multivariate Tobit and logistic regression models were applied to examine factors associated with the severity of depressive symptoms and medication adherence

Results. Approximately 45.2% of participants were identified as having depressive symptoms at different levels. The proportion of patients with mild, moderate, moderately severe, and severe depressive symptoms is 36.0%, 7.6%, 1.4%, and 0.2% respectively. Regarding the treatment process, patients being treated for their diabetes for a longer time were more likely to have depressive symptoms. Depression was positively linked to currently drinking alcohol (Coef = 1.04; 95% CI = 0.30-1.78), having comorbidities (Coef = 1.08; 95% CI = 0.15; 2.01) and having irregular physical activities (Coef = -1.28; 95% CI = -2.18; -0.38). Patients with severe depressive symptoms (higher PHQ-9 score) were more likely to be non-adherent to their medications in the last month (AOR = 1.30; 95% CI = 1.17; 1.46).

Conclusion. Our study shows that a high percentage of patients with diabetes have depressive symptoms. There is a strong association between having depressive symptoms and non-adherence to medications in the last month. To reduce the risk of developing depressive symptoms, depression should be screened at the initial treatment process and patients should be advised to avoid alcohol and to engage in physical activities regularly.

Key words: diabetes, depression, depressive symptom, medication adherence, Vietnam

INTRODUCTION

Diabetes is considered a major public health concern which is increasing dramatically in all countries. The estimated number of individuals with diabetes currently stands at 537 million, with projections indicating that this figure will rise to 643 million by 2030 and further increase to 783 million by 2045.[1] Nearly 80% of those with diabetes reside in low- and middle-income countries (LMICs), where the prevalence is rising rapidly. About 90% of those with diabetes have type 2 diabetes.[2,3] Between 2000 and 2019, there was a 3% increase in diabetes mortality rates by age.[4] During the year 2019, diabetes directly resulted in 1.5 million deaths, with nearly half (48%) of those deaths occurring before individuals reached the age of 70.[5]

Diabetes and psychiatric disorders often co-occur. The prevalence of depression in persons with diabetes is two times higher than that of the general population,[6-8] and share a bidirectional relationship. However, psychiatric disorders are commonly undiagnosed or underestimated among people with diabetes.[9] People with mental health problems are more likely to experience unhealthy lifestyles (overeating or physical inactivity) which adds to the development of diabetes.[6] Meanwhile, the necessity for persons with diabetes to strictly adhere to medication

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and self-care regimens, and the costs associated with medical care, may lead to psychological impairments.[10-12] Both mental disorders and diabetes may negatively affect the quality of life of patients with diabetes.[13] Where co-morbidity occurs, it presents significant clinical challenges and worsens the health outcomes of patients.[14]

The prevalence of psychological problems among patients with diabetes varies across settings. A study assessing depressive symptoms using the Geriatric Depression Scale in elderly patients with diabetes in the National Geriatric Hospital, Hanoi, Vietnam revealed that up to 80% of patients had depressive symptoms,[15] whereas only 30.6% of a Japanese sample of elderly patients with diabetes, screened with the Patient Health Questionnaire 9 (PHQ-9)reported depressive symptoms.[16] Furthermore, a cross-sectional study from India using the PHQ-9 found that 18% of the participants suffered from moderate (PHQ-9 range from 10 – 14) or severe depression (PHQ-9 ≥20).[17]

During the last decade, the prevalence of diabetes in Vietnam has nearly doubled, to an alarming level. New diagnoses were most common in young people in urban areas.[18] In 2016, one in every 20 people was diagnosed with diabetes, and diabetes was more prevalent in urban than in rural areas.[19] However, there is little data regarding depressive symptoms among people with diabetes. Therefore, this study aims to explore the level of depressive symptoms and its associated factors among patients with type 2 diabetes mellitus in Hanoi, Vietnam. This is the first and critical step towards priority setting, implementation, and assessment of a psychological intervention program for patients with diabetes in the community.

METHODOLOGY

Subjects

A cross-sectional study was conducted involving outpatient diabetes patients that were under treatment at the Family Medicine Center (FMC) of the Agricultural General Hospital, Hanoi, Vietnam, one of the largest epicenters that offers health services for patients with diabetes.[20]

The inclusion criteria were (1) Age 18 years old or above; (2) Diagnosed with type 2 diabetes at least 6 months prior to the study, according to the "Guidelines for Diagnosis and Treatment of Type 2 Diabetes" issued by the Vietnamese Ministry of Health in 2017 (Decision No. 3319/QD-BYT);[21] (3) Registered as an outpatient receiving treatment for diabetes in the Department of Outpatient of Agricultural General Hospital; (4) Able to read, write and communicate with the interviewer. We excluded patients who had severe disease conditions or refused to take part in the study. Eligible patients were asked for written informed consent before participating in the study.

The sample size was based on the findings of Hermanns et al., with an estimated 14.1% of patients with diabetes having depressive symptoms. With an $\alpha = 0.05$ and $\varepsilon = 0.03$, the required sample size was computed to be 517 patients. Anticipating refusal to participate in the study, we invited 519 patients. Convenience sampling was done from the lists of patients who were scheduled for a routine follow-up appointment.

Materials and methods

Following their scheduled routine health appointments, patients were invited to fill out a 20-minute questionnaire, consisting of the following segments: Sociodemographic characteristics such as gender, age, occupation and educational level, and Diabetes treatment-related and health risk behavior characteristics.

Participants were asked about the duration of their diabetes, comorbidities including hypertension, dyslipidemia, heart diseases and lung diseases, alcohol consumption, smoking behaviors, and physical activity.

Patient Health Questionnaire (PHQ-9)

To assess the severity of depressive symptoms among patients with diabetes, the Patient Health Questionnaire (PHQ-9) was used. This tool was formulated based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria and the Vietnamese version was shown to be highly valid and reliable.[22] The questionnaire has nine items, and each item may be scored from 0 to 3; therefore, the total score may range from 0 to 27.

The following cut-offs were used to classify depression severity: 0–4 points is normal, 5–9 points is mild, 10–14 points is moderate, 15–19 points is moderate-severe, and ≥20 points is severe. Patients with scores higher than 4 were considered as "having depressive symptoms."[23]

The questionnaire was piloted on 20 volunteers (also outpatient diabetes), resulting in minor changes in the wording of diabetes treatment-related characteristics. During the study, the questionnaire was administered by a final-year nursing student, who had been well-trained regarding the study objectives, the questionnaire, and how to collect data.

Statistical analysis

Multivariate Tobit and logistic regression models were applied to examine factors associated with the severity of depressive symptoms and medication adherence. To identify the reduced regression model, we added a forward stepwise selection strategy with a threshold of less than 0.2. A *p*-value less than 0.05 was considered statistically significant. Pearson's chi-square test was used for categorical variables, and the Mann-Whitney U test was used for non-parametric continuous variables for comparing demographic characteristics, and health risk behaviors of participants between those who have and those who do not have depressive symptoms. All data analyses

were done using STATA software version 12 (Stata Corp. LP, College Station, United States of America).

Ethics approval

Ethics approval was obtained from the Institutional Review Board of Hanoi University of Public Health number 316/2021/YTCC-HD3 on the 12th of July 2021.

RESULTS

The socioeconomic characteristics of patients with diabetes are presented in Table 1. Of the 519 type 2 out-patients with diabetes invited to the study, 516 (99.4%) participated. Most participants finished secondary school (48.6%), retired (63.2%), and enrolled in a compulsory public health insurance scheme (78.7%). There were 231 patients (44.8%) with depression symptoms, ranging from mild to severe levels (PHQ-9 ≥5).

While the study could not find a significant difference in the educational level and occupation of surveyed patients with diabetes, health insurance schemes of the two groups were significantly different.

Patients with diabetes with depressive symptoms were older and lived farther from health facilities than patients without depressive symptoms ($p<.05$). The mean age of the whole sample size was 67 years old (SD = 9.0) and those who were depressed were statistically significantly older than those who did not have depression (68 and 66 years old respectively). The distance to health facilities between the two groups showed the same result, and the mean distance was 5.4 km (SD = 5.1).

Table 2 highlights the clinical-related symptoms and health risk behaviors of patients with diabetes. About 10% and more than 15% of participants currently smoked and drank alcohol respectively. The majority of the participants (84.0%) engaged in daily physical activities. The prevalence of comorbidities was high (90.3%) and 16.7% of patients reported having side effects from their medications within the last 3 months. Regarding disease symptoms, 17.1% of patients reported headache, dizziness and about one-third had numbness of the limbs. Approximately 10% of the participants reported forgetting to take their medications within the last month. The mean duration of the disease was 8.0 years (SD = 6.5) and the mean BMI was 22.8 (SD = 2.8). Only alcohol drinking was found to be significantly different between groups.

Table 3 indicates the depressive symptom characteristics of patients with diabetes. About 36.0% of participants were classified as having mild depressive symptoms, while 10% scored worse (PHQ-9 ≥10). The percentage of patients experiencing moderate, moderate-severe and severe depressive symptoms were 7.6%, 1.4% and 0.2%, respectively. The mean score of PHQ-9 was 5.0 (SD = 2.9), which is in the range of 'mild' symptoms.

Figure 1 depicts the mean score of PHQ-9 plotted against disease duration. The mean PHQ score was relatively constant (i.e., 5 points) among those having diabetes for 0-20 years but gradually increased thereafter.

The results of the multivariate regression models to identify factors associated with having depressive symptoms are shown in Table 4. Older people have more depressive symptoms (Coef = 0.05; 95% CI = 0.01-0.08). Patients who currently drink alcohol were more likely to have higher depressive symptoms compared to those who never use alcohol (Coef = 1.04; 95% CI = 0.30-1.78). In terms of physical activity, exercising several times a week or every day was also associated with a lower score of PHQ-9 (Coef = -0.96; 95% CI = -2.33; -0.42 and Coef = -1.28; 95% CI = -2.18; -0.38, respectively). By contrast, participants with

Table 1. Socio-economic characteristics

	Having depressive symptoms						*p*
	Yes		No		Total		
	n	%	n	%	n	%	
Total	231	44.8	285	55.2	516	100	
Gender							
Male	95	41.1	125	43.9	220	42.6	0.53
Female	136	58.9	160	56.1	296	57.4	
Educational level							
Under secondary school	44	19.1	49	17.2	93	18.0	0.42
Secondary school	117	50.7	134	47.0	251	48.6	
Higher than secondary school	70	30.3	102	35.8	172	33.3	
Occupation							
Employed	70	30.3	93	32.6	163	31.6	0.30
Unemployed	16	6.9	11	3.9	27	5.2	
Retired	145	62.8	181	63.5	326	63.2	
Health insurance							
Obligation	172	74.5	234	82.1	406	78.7	0.04*[a]
Volunteer	59	25.5	51	17.9	110	21.3	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p***
Age	68	9.0	66	8.0	67	9.0	0.03*[b]
Distance to health facility (km)	5.6	5.4	5.2	4.7	5.4	5.1	0.03*[b]

Note: Italicized values are significant at *p <0.05; [a] from Pearson's chi-squared test p-value; [b] from Mann-Whitney U test p-value for non-parametric variables.

Table 2. Clinical-related symptoms and health risk behavior

	Having depressive symptoms						*p*
	Yes		No		Total		
	n	%	n	%	n	%	
Smoking							0.27
Never smoking	186	80.5	214	75.1	400	77.5	
Former smoking	23	10.0	41	14.4	64	12.4	
Currently smoking	22	9.5	30	10.5	52	10.1	
Drinking alcohol							0.04*[a]
Never drinking	186	81.9	206	72.8	392	76.9	
Former drinking	13	5.7	21	7.4	34	6.7	
Currently drinking	28	12.3	56	19.8	84	16.5	
Physical activity							0.27
Several times per month	24	8.8	28	13.2	52	10.7	
Several times per week	16	5.8	10	4.7	26	5.3	
Everyday	234	85.4	175	82.2	409	84.0	
Other people in family having diabetes	63	22.7	53	23.4	116	23.0	0.86
Comorbidities	208	88.1	174	93.1	382	90.3	0.09
Disease symptoms							
Unexplained weight loss	14	4.9	18	7.8	32	6.2	0.18
Headache, dizzy	55	19.3	33	14.3	88	17.1	0.13
Numbness of the limbs	102	35.8	73	31.6	175	33.9	0.32
Forgot to take pills last month	22	8.2	24	11.2	46	9.5	0.28
Side effect of medicine within last 3 months	42	14.7	44	19.1	86	16.7	0.19
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p***
Duration of disease (year)	8.6	6.9	7.6	6.1	8.0	6.5	0.13
BMI	22.8	2.7	22.9	2.8	22.8	2.8	0.70

Note: Italicized values are significant at *p<0.05; [a] from Pearson's chi-square test p-value

Table 3. Level of depressive symptoms among diabetic patients

	Frequency (n)	Percentage (%)
Normal	283	54.8
Mild	186	36.0
Moderate	39	7.6
Moderate severe	7	1.4
Severe	1	0.2
	Mean	**SD**
PHQ-9 score	5.0	2.9

Table 4. Factors associated with having depressive symptoms among diabetic patients

	PHQ9 score	
	Coef	95% CI
Age	0.05**	0.01; 0.08
Drinking alcohol (vs never)		
Currently drinking	1.04**	0.30; 1.78
Physical activity (vs several times a month)		
Several times a week	-0.96*	-2.33; -0.42
Everyday	-1.28**	-2.18; -0.38
Comorbidities	1.08*	0.15; 2.01

Note: Italicized values are significant at *p<0.05, **p<0.01.

comorbidities had a higher likelihood of having depressive symptoms than those who did not (Coef = 1.08; 95% CI = 0.15; 2.01).

Table 5 shows the factors associated with medication adherence among patients with diabetes. Notably, patients having more severe depressive symptoms (higher PHQ-9 scores) were more likely to forget taking their medication in the last month (AOR=1.30; 95% CI = 1.17; 1.46). Being female and being older was associated with non-adherence to medication in the previous month (AOR=0.45; 95% CI =

Table 5. Factors associated with medication adherence among diabetic patients

	Missed medication last month (Yes vs No)	
	AOR	95%CI
Gender (female vs male)	0.45*	0.20; 0.50
Age	0.95*	0.91; 0.99
Health insurance (volunteer vs obligation)	0.35*	0.12; 0.99
Drinking alcohol (vs never)		
Currently drinking	1.41*	1.13; 1.51
Depressive symptom	1.30**	1.17; 1.46
Numbness of the limbs (yes vs no)	1.48*	1.17; 1.60
Side effect of medicine within last 3 months (yes vs no)	2.63**	1.10; 6.30

Note: Italicized values are significant at *p<0.05, **p<0.01.

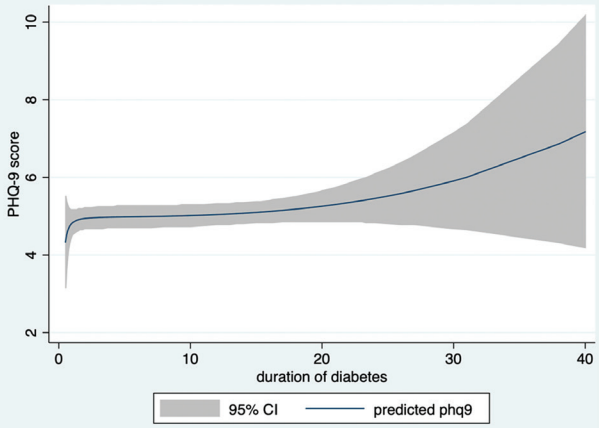

Figure 1. The mean of PHQ-9 score for the duration of diabetes.

0.20; 0.05 and AOR=0.95; 95% CI = 0.91-0.99, respectively). By contrast, those who currently drink alcohol, have numbness of the limbs, and experienced side effects from medicine within the last 3 months had a lower likelihood of sufficiently adhering to medication.

DISCUSSION

Our study provided valuable evidence on the level of depression among diabetes patients in a primary setting in Vietnam. We found that nearly half of the patients suffered from mild to severe depressive symptoms. Moreover, depressive symptoms increased gradually from the early to the late stage of the treatment process. Currently drinking alcohol and having comorbidities were positively associated with more severe depression while doing physical activities related to lower PHQ-9 scores. Notably, patients with a higher level of depressive symptoms were more likely to forget to take their medicine in the last month.

The percentage of participants having depressive symptoms in our study was lower than that of other studies in Vietnam[15] and in other countries.[16-24] The difference can be explained by the variation of individual characteristics in our sample. We enrolled patients with diabetes aged 18 and above, while other studies collected data on elderly patients only. When compared to patients having chronic diseases such as cardiovascular disorders, results from our study are similar to previous studies,[25,26] Diabetes and cardiovascular disease are the most common chronic diseases,[27] for which patients have to undergo long treatment duration and strict compliance to a treatment regimen.

Regarding treatment duration, we found that patients who had long-standing diabetes were more likely to have depressive symptoms. At the start of the treatment process, they likely had to adapt to rigorous treatment procedures, which might have contributed to the development of depressive symptoms.[28-29] Moreover, patients with diabetes have to live with the condition for the rest of their lives,[30] therefore, they are more likely to have more severe depressive symptoms later in the treatment process.

Our study also emphasized aging in mental health problems. As prolonged diabetic treatment has been positively associated with an accelerated aging process, age-related comorbidities may reduce brain function and trigger several symptoms of depression.[15] Thus, an intervention that reduces depressive symptoms among patients with diabetes should focus on elderly patients or patients at the later stages of treatment.

The findings from our study are similar to several previous research in the association between persons with diabetes using alcohol and having depressive symptoms.[31] Heavy alcohol use is more common in those who are more depressed than among the general population.[32] On the other hand, there is evidence that chronic alcohol use causes high depressive symptoms.[33]

The study also revealed that having comorbidities were related to having depressive symptoms. Given that heavy alcohol use may increase the risk of having cardiovascular comorbidities, individuals who have both problems may have a higher risk of depression.[34]

In contrast, physical activity was associated with a lower level of depressive symptoms. Regular exercise is considered to have a positive effect in preventing the development of chronic diseases, as well as decreasing the likelihood of depression and alleviating mental stress.[35] Among patients with diabetes, physical activity also supports maintaining normal glucose uptake which contributes to better glucose control.[36]

Notably, a higher level of depressive symptoms was related to a higher likelihood of not taking medicine. This is in line with previous studies exploring the impact of depression on adherence to diabetic medication.[10,37] Compared with other patients, persons with diabetes were at a higher risk of experiencing depression and anxiety disorders.[38] Moreover, stressors and depressive symptoms are associated with medication nonadherence.[39] To reduce the long-term complications of diabetes, continuous medical care, self-management, and adherence to prescribed medication are required.[40] Therefore, earlier assessment of mental health problems among patients with diabetes will limit adverse effects and reduce the risk of long-term complications during the treatment process.

Several implications can be drawn from our study. As the level of depressive symptoms was found to be associated with non-adherence to medication, a comprehensive health-care program should integrate physical and psychological examination. Screening and intervention for depression should be initiated early in the treatment process to minimize the development of depressive symptoms later in the disease course, alongside limiting complications and providing psychological support. Avoiding alcohol and engaging in regular physical activities play an essential role in reducing the risk of developing depressive symptoms.

Nevertheless, our study has some limitations. First, self-reported information may lead to recall bias and social desirability bias. In order to minimize these, we excluded interviewers who were affiliated with the hospital. In addition, the instructions, as well as the purposes of the study, were explained clearly to the participants. Second, the cross-sectional study design may reduce the causal inferences between having depressive symptoms and non-adherence to medication. Finally, the generalizability of study results to other international populations can be limited due to the convenience sampling technique.

CONCLUSION

Our study reveals that a great number of patients with diabetes have depressive symptoms. There is a strong association between having depressive symptoms and

non-adherence to medication in the last month. To reduce the risk of developing depressive symptoms, depression should be screened for during the initial treatment process and patients should be advised to avoid alcohol and engage in regular physical activities.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
KTN: Conceptualization, Methodology, Software, Formal analysis; Writing – original draft preparation; **HPN:** Conceptualization, Methodology, Writing – original draft preparation; **KVB:** Writing – review and editing; **JW:** Conceptualization, Methodology, Writing – original draft preparation.

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ORIGINAL ARTICLE

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Association of Acute Hyperglycemia and Diabetes Mellitus with Platelet-derived Microparticle (PDMP) Levels During Acute Myocardial Infarction

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Abstract

Objectives. This research investigates whether there is an association between acute hyperglycemia and diabetes mellitus and the level of circulating platelet-derived microparticles (PDMPs) during an initial episode of acute myocardial infarction (AMI).

Methodology. This was a cross-sectional study involving hospitalized AMI patients. Demographic and clinical data were obtained from hospital records. Diabetes mellitus was defined by the history of the disease, anti-diabetes medication use and/ or level of HbA1c ≥6.5%. Levels of HbA1c, admission random and fasting blood glucose levels were measured. Flow-cytometry method was used to determine the levels of PDMPs from collected venous blood through tagging with CD-41 FITC and CD-62 PE markers and a threshold size of <1 μm. The number of circulating PDMPs was compared according to glucometabolic state, namely acute hyperglycemia (admission random glucose ≥200 mg/dL and fasting glucose ≥140 mg/dL) and diabetes mellitus. The comparative analysis between groups was conducted with Student T-test or Mann-Whitney test, where applicable.

Results. A total of 108 subjects were included and their data analyzed. The level of circulating PDMPs was significantly lower in subjects with admission random glucose ≥200 mg/dL as compared to those with below level [median (interquartile range (IQR)]: 2,710.0 (718.0-8,167.0) count/mL vs. 4,452.0 (2,128.5-14,499.8) count/mL, $p = 0.05$) and in subjects with fasting glucose ≥140 mg/dL as compared to those with below level (median (IQR): 2,382.0 (779.0-6,619.0) count/mL vs. 5,972.0 (2,345.7-14,781.3) count/mL, $p = 0.006$). The level of circulating PDMPs was also significantly lower in patients with diabetes mellitus as compared to those without (median (IQR): 2,655.0 (840.0-5,821.0) count/mL vs. 4,562.0 (2,128.5-15,055.8) count/mL; $p = 0.007$).

Conclusion. Acute hyperglycemia and diabetes mellitus are significantly associated with a lower circulating PDMP level during an initial AMI episode.

Key words: hyperglycemia; diabetes mellitus; cell-derived microparticles; acute myocardial infarction

INTRODUCTION

Cardiovascular diseases (CVD) contribute to the highest number of deaths internationally, amounting to around one-third of all causes of death. Among CVDs, roughly 7.4 million deaths are due to coronary artery disease (CAD), a disorder of the blood vessels supplying the myocardia. CAD is the most common cause of acute myocardial infarction (AMI), which is a cardiac emergency that arises when blood flow to the myocardia is blocked due to an occlusive plaque with subsequent thrombosis in the coronary arteries.[1] It causes the flow of oxygen-rich blood to diminish triggering a reduction in the oxygen supply to the myocardia which results in its injury and infarction.[1]

Diabetes mellitus and hyperglycemia are among the risk factors for CVD.[2] Hyperglycemia is encountered in up to 50% of patients with AMI, especially in ST-elevation acute myocardial infarction (STEMI).[3]

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Diabetes mellitus, a disease characterized by chronic hyperglycemia, is an important risk factor for AMI and exists in about 30% of AMIs.[3] Our previous studies showed that in patients with acute hyperglycemia and diabetes mellitus, higher levels of blood glucose during AMI episodes increased the incidence of adverse cardiovascular events.[4,5] One mechanism that may explain the poorer prognosis is the enhanced platelet activation and subsequent thrombus formation.[6]

Platelet-derived microparticles (PDMPs) play an important role in CVD, especially in atherosclerotic CVD. They are small vesicles formed from platelet plasma membranes and released during platelet activation or apoptosis through exosome exocytosis and budding of the plasma membrane.[7] Our previous studies indicated that PDMP amounts increased during AMI episodes and are associated with worse short and long-term outcomes.[8,9]

In patients with diabetes mellitus, the PDMPs increased significantly compared to the normal population.[10] Furthermore, in patients with diabetes with CVD, the PDMPs were multiplied, indicating platelet activation in this population.[11] During an AMI episode, more enhanced platelet activation and subsequent platelet-enriched thrombus formation among patients with diabetes mellitus may amplify the production of PDMPs. However, despite the abundant data on increased circulating PDMPs among diabetes mellitus and stable CVD, the impact of acute hyperglycemia and diabetes mellitus during AMI on circulating PDMPs release has not yet been investigated. Therefore, circulating PDMPs might be used as a biomarker to assess advanced intracoronary thrombosis in acute hyperglycemia and diabetes mellitus patients during an AMI episode.

This research investigates the association between acute hyperglycemia and diabetes mellitus and circulating PDMP levels during the initial episode of AMI.

METHODOLOGY

This research used a cross-sectional methodology with 108 patients enrolled in the study. The included patients had AMI, both ST and non-ST segment elevation myocardial infarction (STEMI and NSTEMI), who were admitted and underwent hospitalization in the Intensive Cardiac Care Unit of Dr. Sardjito Hospital, Yogyakarta from January 2017 to January 2019. The AMI diagnosis was based on three parameters: anginal pain, abnormal electrocardiogram findings and increased level of troponin-I. An initial episode of AMI was defined as within 24 hours of hospital admission.

Specifically, the inclusion criteria were: (1) Diagnosis of STEMI and NSTEMI; (2) Chest pain onset <24 hours; (3) Age between 35 and 75 years old; and (4) Fee of chronic comorbidities (chronic renal failure, congestive heart failure, and hepatic cirrhosis).

The exclusion criteria were: (1) Unmeasured or missing random and fasting blood glucose levels; (2) Unmeasured or missing HbA1c level;(3) Concurrent acute infection and sepsis; (4) Concurrent acute stroke; and (5) Co-existing malignancies.

Demographic and clinical data were obtained from hospital records and collected into case report forms. Diabetes mellitus was determined from the patients' history of the disease, medical history of anti-diabetes medication use and/ or level of HbA1c ≥6.5%. Other risk factors were defined accordingly. Within 24 hours of admission, venous blood samples were taken for laboratory examination. Hematology parameters of hemoglobin, leukocytes and platelets, including HbA1c, were examined with an automated hematocytometer based on the hospital standard (Sysmex XN1000®, Sysmex, Kobe, Japan). The examination of admission random glucose and fasting glucose levels employed the hexokinase method (Cobas 6000® analyzer, Roche Diagnostic, Mannheim, Germany). Other chemical examinations were performed with a chemical analyzer in our hospital laboratory.

As previously described, the level of circulating PDMPs was measured through flow-cytometry method (FACS Calibur, Becton Dickinson, Maryland, USA) within 24 hours of admission. The PDMPs were identified by tagging with CD-41 FITC (Biolegend, San Diego, USA) and CD-62 PE markers (Biolegend, San Diego, USA). The threshold size of <1 μm was defined and the amount was gated and calculated with the standard formula as described previously.[8]

Based on admission random glucose level, subjects were divided into acute hyperglycemia (admission random glucose level ≥200 mg/dL) and those without acute hyperglycemia (admission random glucose level <200 mg/dL). Based on fasting glucose level, subjects were divided into fasting hyperglycemia (fasting glucose level ≥140 mg/dL) and those without fasting hyperglycemia (fasting glucose level <140 mg/dL).

The subjects were also divided based on their history of diabetes mellitus. The calculation of sample size was based on the comparative study, namely the sample size formulae for comparing two independent groups (acute hyperglycemia and diabetes mellitus versus none of the conditions) with continuous variables (circulating PDMP levels) as previously described.[12] With the type one error of 5% and type two error of 80%, each group's minimum sample size was computed at 45 subjects.

All subjects completed and signed an informed consent authorization to participate in this research. Universitas Gadjah Mada Medical and Health Research Ethics Committee and the Dr. Sardjito Hospital Institutional Review Board approved the research protocol with number: (KE/FK/720/EC/2016).

Statistical analysis

The comparison of numerical variables, including PDMP levels, between groups (with hyperglycemia versus without hyperglycemia and with diabetes mellitus versus without diabetes mellitus) was analyzed with Student T-tests or Mann-Whitney tests, where applicable, after normality distribution analysis with Kolmogorov-Smirnov test. The comparison of categorical variables utilized the Chi-square test or Fisher's exact test. The correlations between circulating PDMPs amounts and glucose levels as well as HbA1c were analyzed with Spearman's correlation test. The results with p-value <0.05 were considered significant. The statistical analysis was performed with the SPSS software version 25.0 (IBM Corp., Armonk, NY, U.S.A).

RESULTS

Subject characteristics

During the study period, 120 patients were admitted with AMI. Among these 120 patients, 108 subjects were eligible for analysis in this research, while 12 subjects were excluded due to lacking glucose or HbA1c data. Of the included AMI patients, 15 (13.9%) were NSTEMI and 93 (86.1%) were STEMI (Figure 1).

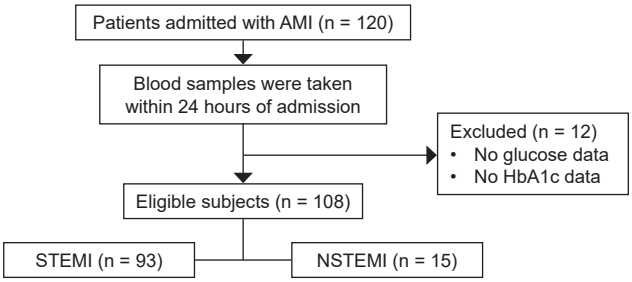

Figure 1. Study flowchart.

Admission blood glucose and circulating PDMPs amount

Patients with admission glucose level ≥200 mg/dL (n = 23, 21%) were observed to have a significantly higher percentage of diabetes mellitus (69.6%), STEMI diagnosis (100%), higher level of fasting glucose (mean: 187.8 ± 73.2 mg/dL) and higher concentration of HbA1c (mean: 9.1±2.3%), as compared to subjects with admission glucose level <200 mg/dL (n = 85, 79%) (Table 1).

The circulating PDMP level was significantly lower in subjects with admission glucose level ≥200 mg/dL as compared to those with below level (median interquartile range (IQR): 2,710.0 (718.0-8,167.0) count/mL vs. 4,452.0 (2,128.5-14,499.8) count/mL, p = 0.05).

Fasting glucose and circulating PDMPs amount

Patients with fasting glucose level ≥140 mg/dL (n = 33, 31%) also had a significantly higher percentage of diabetes mellitus (69.7%), lower level of leukocyte counts (mean: 11.6 ± 2.9 x 10^3/mm^3), higher level of admission glucose (mean: 224.1 ± 81.2 mg/dL) and higher percentage of HbA1c (mean: 8.6 ± 2.3%) as compared to patients with fasting glucose level <140 mg/dL (n = 75, 69%) (Table 2).

The circulating levels of PDMP were significantly lower in patients with fasting glucose level ≥140 mg/dL as compared to those with below level (median (IQR): 2,382.0 (779.0-6,619.0) count/mL vs. 5,972.0 (2,345.7-1,4781.3) count/mL, p = 0.006).

Diabetes mellitus and circulating level of PDMP

Among the patients diagnosed with diabetes mellitus (n = 27, 25%) compared to those without, there were fewer

Table 1. The characteristics of subjects based on admission random glucose level cut-off value of 200 mg/dL

Characteristics	Admission glucose level <200 mg/dL (n = 85)	Admission glucose level ≥200 mg/dL (n = 23)	p
Age (years), mean ± SD	54.9 ± 8.4	55.4 ± 8.8	0.41
Males, n (%)	79 (92.9)	19 (82.6)	0.13*
Females, n (%)	6 (7.1)	4 (17.4)	0.13*
Hypertension, n (%)	42 (49.4)	11 (44.1)	0.15
Diabetes mellitus, n (%)	11 (12.9)	16 (69.6)	<0.001
Dyslipidemia, n (%)	21 (24.7)	5 (4.6)	0.77
Ischemic heart disease, n (%)	24 (28.2)	6 (26.1)	0.84
Current smoker, n (%)	52 (61.2)	8 (34.8)	0.11
Bodyweight (kg), mean ± SD	65.5 ± 12.9	64.2 ± 12.0	0.33
Body mass index (kg/m^2), mean ± SD	26.5 ± 19.1	24.5 ± 3.1	0.31
STEMI, n (%)	70 (82.4)	23 (100.0)	0.02*
NSTEMI, n (%)	15 (17.6)	0 (0)	0.02*
Hemoglobin (g/dL), mean ± SD	13.8 ± 1.6	14.2 ± 1.7	0.11
Leukocytes (x10^3/mm^3), mean ± SD	12.9 ± 3.6	12.4 ± 3.4	0.24
Platelets (x10^3/mm^3), mean ± SD	265.6 ± 78.8	277.4 ± 89.7	0.27
HbA1c (%),* mean ± SD	6.1 ± 1.1 (n = 78)	9.1 ± 2.3 (n = 20)	<0.001
Fasting blood glucose (mg/dL), mean ± SD	117.1 ± 27.2	187.8 ± 73.2	<0.001
Urea nitrogen (mg/dL), mean ± SD	15.4 ± 7.5	16.8 ± 5.8	0.21
Creatinine (mg/dL), mean ± SD	1.2 ± 0.4	1.2 ± 0.4	0.48
PDMPs (count/uL), median (IQR)	4,452.0 (2128.5-14,499.8)	2,710.0 (718.0-8,167.0)	0.05

STEMI: ST-segment elevation acute myocardial infarction; NSTEMI: non-ST-segment elevation acute myocardial infarction; HbA1c: glycated hemoglobin; PDMPs: platelet-derived microparticles; SD: standard deviation; IQR: interquartile range. *Fisher-exact test.

males (77.8% versus 95.1%); lower percentage of current smokers (37% versus 61.7%);higher mean level of admission glucose (231.7 ± 76.9 mg/dL versus 138.7 ± 50.1 mg/dL); higher mean level of fasting glucose (187.1 ± 56.1 mg/dL versus 113 ± 31.6 mg/dL) and higher HbA1c percentages (8.6 ± 2.2% versus 6.1 ± 1.3%). (Table 3).

The circulating level of PDMP was significantly lower in patients with diabetes mellitus as compared to those without diabetes mellitus (median (IQR): 2,655.0 (840.0-5,821.0) count/mL vs. 4,562.0 (2,128.5-15,055.8) count/mL; $p = 0.007$).

Correlation between blood glucose and HbA1c levels and circulating PDMPs amount

The correlation test indicated that circulating level of PDMPs had a significantly negative correlation with fasting blood glucose (r = -0.213; $p = 0.03$). However, no significant correlations were found with admission blood glucose (r = -0.141; $p = 0.15$) and concentration of HbA1c (r = -0.059; $p = 0.57$), as shown in Table 4.

DISCUSSION

The research findings indicate that patients with acute hyperglycemia during AMI, defined by admission random blood glucose ≥200 mg/dL and fasting blood glucose ≥140 mg/dL, had lower levels of circulating PDMP. Furthermore, patients with a chronic hyperglycemia state, as in diabetes mellitus, were noted to have lower levels of circulating PDMP measured during the AMI episode. A negative correlation was found between glycemic indices and circulating PDMP levels, with significant findings only for fasting blood glucose levels.

Based on our study, patients with acute hyperglycemia had lower levels of circulating PDMP in comparison to those without acute hyperglycemia. Furthermore, during the fasting state, those with persistent acute hyperglycemia had significantly lower levels of circulating PDMP, when measured during the acute phase of AMI.

In previous studies, patients with diabetes mellitus had higher circulating PDMP than subjects without diabetes mellitus because there is an enhanced endothelial dysfunction and platelet hyperactivity in diabetes mellitus.[13] Plasma circulating PDMPs had significant and positive correlations with both fasting blood glucose and HbA1c levels.[14] Among patients with diabetes mellitus, significantly elevated levels of PDMPs were found also in patients with microvascular and macrovascular complications and associated with the severity of microvascular complications.[15,16] In the long term, PDMPs participate in atherosclerosis formation and progression by upregulating cytokines and intercellular adhesion molecular-1, facilitating leukocyte migration and hampering nitric oxide (NO) production.[17]

In a meta-analysis, diabetes mellitus was significantly associated with increased levels of PDMPs.[10] However, these previous findings were investigated in diabetes mellitus without an acute thrombosis event, such as AMI. In our study, which was comprised of subjects with AMI, the subjects with diabetes mellitus did not have a significant increase in PDMP levels. In contrast to previous findings, these patients had lower levels as compared to those without diabetes.

During AMI, PDMP levels increase especially at the beginning of the disease.[18] The increasing level of PDMPs was significantly associated with platelet activation, a vicious cycle of inflammation and thrombosis, the ischemic burden of myocardia and worsened clinical outcomes.[10,14,18]

In this current research, the circulating PDMPs were reduced in acute hyperglycemia and diabetes mellitus

Table 2. The characteristics of subjects based on fasting glucose level cut-off value of 140 mg/dL

Characteristics	Fasting glucose level <140 mg/dL (n = 75)	Fasting glucose level ≥140 mg/dL (n = 33)	*P*
Age (years), mean ± SD	54.6 ± 8.8	56.1 ± 7.5	0.20
Males, n (%)	71 (94.7)	27 (81.8)	0.04*
Females, n (%)	4 (5.3)	6 (18.2)	0.04*
Hypertension, n (%)	35 (46.7)	18 (53.0)	0.29
Diabetes mellitus, n (%)	4 (5.3)	23 (69.7)	<0.001
Dyslipidemia, n (%)	17 (22.7)	9 (27.3)	0.61
Ischemic heart disease, n (%)	18 (24.0)	12 (36.4)	0.19
Current smoker, n (%)	47 (62.7)	13 (39.4)	0.17
Bodyweight (kg), mean ± SD	64.7 ± 12.7	66.4 ± 12.8	0.26
Body mass index (kg/m^2), mean ± SD	24.1 ± 4.2	25.2 ± 3.2	0.11
STEMI, n (%)	67 (89.3)	26 (78.8)	0.14
NSTEMI, n (%)	8 (10.7)	7 (21.2)	0.14
Hemoglobin (g/dL), mean ± SD	13.8 ± 1.6	13.9 ± 1.7	0.45
Leukocytes (x10^3/mm^3), mean ± SD	13.4 ± 3.7	11.6 ± 2.9	0.01
Platelets (x10^3/mm^3), mean ± SD	272.6 ± 87.5	257.8 ± 66.9	0.19
HbA1c (%)*, mean ± SD	6.0 ± 0.8 (n = 70)	8.6 ± 2.3 (n = 28)	<0.001
Admission blood glucose (mg/dL), mean ± SD	134.7 ± 42.8	224.1 ± 81.2	<0.001
Urea nitrogen (mg/dL), mean ± SD	15.3 ± 7.2	16.4 ± 7.4	0.25
Creatinine (mg/dL), mean ± SD	1.3 ± 0.5	1.1 ± 0.4	0.08
PDMPs (count/uL), median (IQR)	5,972.0 (2,345.7-14,781.3)	2,382.0 (779.0-6,619.0)	0.006

STEMI: ST-segment elevation acute myocardial infarction; NSTEMI: non ST-segment elevation acute myocardial infarction; HbA1c: glycated hemoglobin; PDMPs: platelet-derived microparticles; SD: standard deviation; IQR: interquartile range. *Fisher exact test

Table 3. The characteristics of subjects based on presence of diabetes mellitus

Characteristics	Diabetes mellitus (n = 27)	Non-diabetes mellitus (n = 81)	*p*
Age (years), mean ± SD	56.0 ± 6.9	54.7 ± 8.9	0.25
Males, n (%)	21 (77.8)	77 (95.1)	0.02*
Females, n (%)	6 (22.2)	4 (4.9)	0.02*
Hypertension, n (%)	17 (63.0)	36 (44.4)	0.09
Dyslipidemia, n (%)	8 (29.6)	18 (22.2)	0.44
Ischemic heart disease, n (%)	11 (40.7)	19 (23.5)	0.08
Current smoker, n (%)	10 (37.0)	50 (61.7)	0.03
Bodyweight (kg), mean ± SD	64.7 ± 13.5	65.4 ± 12.5	0.40
Body mass index (kg/m^2), mean ± SD	24.8 ± 3.7	24.3 ± 4.0	0.32
STEMI, n (%)	22 (81.5)	71 (87.7)	0.42
NSTEMI, n (%)	5 (18.5)	10 (12.3)	0.42
Hemoglobin (g/dL), mean ± SD	13.6 ± 1.7	13.9 ± 1.6	0.17
Leukocytes (x10^3/mm^3), mean ± SD	12.0 ± 2.9	13.1 ± 3.7	0.09
Platelets (x10^3/mm^3), mean ± SD	262.6 ± 67.2	269.9 ± 85.3	0.34
HbA1c (%), mean ± SD	8.6 ± 2.2 (n = 24)	6.1 ± 1.3 (n = 74)	<0.001
Admission blood glucose (mg/dL), mean ± SD	231.7 ± 76.9	138.7 ± 50.1	<0.001
Fasting blood glucose (mg/dL), mean ± SD	187.1 ± 56.1	113.8 ± 31.6	<0.001
Urea nitrogen (mg/dL), mean ± SD	19.4 ± 7.6	14.4 ± 6.7	<0.001
Creatinine (mg/dL), mean ± SD	1.3 ± 0.4	1.2 ± 0.4	0.237
PDMPs (count/uL), median (IQR)	2,655.0 (840.0-5,821.0)	4,562.0 (2,128.5-15,055.8)	0.007

STEMI: ST-segment elevation acute myocardial infarction; NSTEMI: non-ST-segment elevation acute myocardial infarction; HbA1c: glycated hemoglobin; PDMPs: platelet-derived microparticles; SD: standard deviation; IQR: interquartile range. *Fisher exact test

Table 4. Correlation analysis between PDMP level with other variables

Variables	Coefficient correlation	
Age (years), mean ± SD	-0.090	0.36
Bodyweight (kg), mean ± SD	-0.036	0.72
Body mass index (kg/m^2), mean ± SD	-0.087	0.38
Hemoglobin (g/dL), mean ± SD	0.006	0.95
Leukocytes (x10^3/mm^3), mean ± SD	0.144	0.14
Platelets (x10^3/mm^3), mean ± SD	0.177	0.07
Admission blood glucose (mg/dL), mean ± SD	-0.141	0.15
Fasting blood glucose (mg/dL), mean ± SD	-0.213	0.03
HbA1c (%), mean ± SD	-0.059	0.57
Urea nitrogen (mg/dL), mean ± SD	-0.109	0.27
Creatinine (mg/dL), mean ± SD	0.037	0.71

HbA1c: glycated hemoglobin; PDMPs: platelet-derived microparticles; SD: standard deviation

during the initial phase of AMI during which intracoronary thrombosis was ongoing. Previous in-vivo and human studies showed that the growing intracoronary thrombi in AMI utilize PDMPs in culprit sites as adhesion and activation markers, which cause reduced amounts in the circulation or peripheral blood.[19] We suggest that in acute hyperglycemia and diabetes mellitus, more PDMPs are generated and function in the culprit sites rather than circulate in the blood. This may possibly explain the findings generated by this research, that there is a reduction of circulating PDMPs among acute hyperglycemia and diabetic subjects during an acute coronary event.

Reduced PDMPs because of platelet reactivity induced by antiplatelet medications has been reported. The loading dose and continuation of double antiplatelet treatment in AMI contribute to the reduction of PDMPs in circulation.[20,21] Our patients underwent a loading dose of double anti-platelets, which may have affected the level of PDMPs during the acute phase of AMI in both groups.

Our previous study indicated the persistence of PDMPs 30 days after the acute phase of AMI.[9] Another study indicated that microparticles derived from endothelial cells (EDMPs) did not significantly differ between patients with diabetes mellitus who suffered from acute coronary syndrome and those with stable CAD, which indicated that the release of apoptosis-induced EDMPs is increased in diabetes, irrespective of the chronicity of the coronary disease.[22]

The findings of our study suggest that circulating PDMPs may be a potential biomarker for enhanced thrombus formation in AMI among patients with diabetes mellitus and acute hyperglycemia. The reduced circulating PDMPs in the initial phase of AMI reflect exaggerated utilization in the culprit lesion. Therefore, it signifies the need for aggressive management in AMI subjects with diabetes mellitus and acute hyperglycemia such as coronary revascularization, adequate fibrinolysis, adequate antiplatelets and potential use of anticoagulants.

CONCLUSION

Patients with acute hyperglycemia and diabetes mellitus tend to have lower levels of circulating PDMP during an initial episode of AMI. The fasting glucose level was negatively correlated with the level of circulating PDMPs. However, a limitation of this study is the relatively small sample size which warrants further investigation with a larger sample size to verify the results.

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Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
HAI: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – original draft preparation, Visualization; **IP:** Methodology, Validation, Writing – review and editing; **DSM:** Methodology, Validation, Writing – review and editing, Supervision; **ABH:** Conceptualization, Methodology, Software, Formal Analysis, Investigation, Resources, Writing – review and editing, Supervision, Project administration, Funding acquisition.

Author Disclosure
The authors declared no conflict of interest.

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ORIGINAL ARTICLE

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A Survey on Factors Affecting Knowledge and Satisfaction with Care Among Persons with Diabetes Mellitus in an Urban Health Centre and its Outreach Clinics in South India

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Abstract

Objective. To determine the level of knowledge and factors affecting knowledge and satisfaction with diabetes care among persons with diabetes at urban health centre (UHC) and community health worker (CHW)-led outreach clinics (ORC) in South India.

Methodology. A cross-sectional study was carried out using a structured questionnaire. One hundred patients at the UHC and 200 patients at the ORC were included.

Results. Patients with DM of more than eight years, with co-morbidities and maintained on insulin had good knowledge at the UHC. At the ORC, participants who received education beyond the primary level and belonging to non - Hindu religion had higher knowledge. Patients at the ORC experienced better satisfaction in terms of waiting time for appointments, consultation, registration system and counselling. At the UHC, those who received primary education or those with lower educational attainment had better satisfaction. Overall, knowledge (p = 0.03) and satisfaction (p = 0.00001) of diabetes care was better at the ORC than at the UHC.

Conclusions. Our study found better knowledge and satisfaction with diabetes care at the ORC than at the UHC. Whether or not the difference can be attributed to CHW-based clinics in the community needs to be further elucidated.

Key words: community health workers, primary health care, knowledge, satisfaction, diabetes

INTRODUCTION

Diabetes mellitus is a rising epidemic in South-East Asia and in India in particular. In 2021, the worldwide number of people with diabetes has been estimated to be 536.6 million. India has 74.2 million people with diabetes and the number is estimated to rise to 124.9 million by 2045.[1] The International Diabetes Federation reports that one in seven adults with diabetes come from India.[1] The median annual direct and indirect costs for diabetes care in India is estimated at 25.391 INR ($525.5) and 4970 INR ($102.8), respectively.[2]

Comprehensive management of diabetes involves active participation of patients in making significant lifestyle modifications.[3] Medication adherence, treatment compliance, self-monitoring of blood sugar and follow-up with their physicians entail long-term commitment from patients and their families.[4] Adherence is the degree to which the person's behavior is consistent with the recommendations and compliance is the extent to which a patient's behavior matches the prescriber's advice.[5] These self-care behaviors are found to be associated with improved glycemic control and reduction in the incidence of complications.

The cognitive evaluation of whether or not a treatment regimen meets the patient's subjective expectations is termed as treatment satisfaction, which may influence treatment adherence in patients with diabetes.[6] A Palestinian study documented that patients with moderate satisfaction had greater adherence to medication and had better quality of life.[7] Treatment adherence has also been found to be related to patient-provider communication. Poor communication was found to be associated with inadequate medication adherence, particularly for oral hypoglycemics.[8]

Knowledge related to diabetes is important to improve self-care and self-monitoring of blood sugar among patients with diabetes. However, poor knowledge of the

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risk factors for developing diabetes is well documented in Indian literature.[9] An Ethiopian study observed that those who were illiterate and had diabetes for less than five years had low knowledge regarding diabetes, thus affecting their compliance and adherence.[10] Other factors found to be associated with poor knowledge of diabetes include age, marital status, occupation and family history of diabetes.[11] A Bangladesh study identified female gender and lower income as factors contributing to the low level of knowledge regarding diabetes.[12] Of the many interventions, health education is one of the proven cost-effective, scalable interventions to improve the knowledge on diabetes in developing countries.[13]

Diabetes care is a significant part of the ambulatory and in-patient services of the urban health centre (UHC) where this study was conducted. About 40% of the out-patient visits to the UHC comprise of care related to diabetes.[14] Nearly 500 patients were admitted during the years 2020-2021 due to diabetes and diabetes-related problems.[15]

The UHC provides primary and secondary level healthcare services to the low-resource urban communities. Outreach clinics (ORCs) were initiated in 2016 to improve accessibility in the low-income areas of the community. The entire population of the community was enlisted by the community health worker (CHW) assigned to the community. Community-based health education sessions are routinely conducted by the CHW and community health nurse (CHN). One assigned team consisting of a physician, CHN, CHW and social worker (SW) provide healthcare services to a particular community. Besides running the weekly clinics, the CHWs screen asymptomatic patients, assess post-hospitalised patients and those who are not compliant to medications.

This study was undertaken to measure the factors associated with the knowledge and satisfaction related to diabetes care at the UHC and ORCs.

OBJECTIVES

1. To determine the level of knowledge on diabetes among patients with diabetes mellitus attending the UHC and the ORCs.
2. To assess the satisfaction with care among patients with diabetes mellitus attending the UHC and the ORCs.
3. To determine factors associated with adequate knowledge and satisfaction with care among patients with diabetes attending the UHC and the ORCs.

METHODOLOGY

Study setting and design

This was a cross-sectional study done at the UHC and ORCs. The UHC is one of the secondary care service units of a private academic tertiary care center, which serves as the former's referral center. The UHC serves a population of 200,000 hailing from the low-resource urban communities of the town. However, not all patients from these communities seek care at the UHC. The clinical services at the UHC and the ORCs are subsidized by the tertiary care center. The UHC is managed by a team comprising of physicians trained in Family Medicine, Community Medicine, post graduate trainees and junior medical officers.

The UHC has a capacity of 46 inpatient beds, two beds in the labor room and an operating room, and the center also includes laboratory and pharmacy services. It provides daily ambulatory care, weekly antenatal services in addition to the ORCs serving the local urban communities. Approximately 180-200 patients seek healthcare daily in the out-patient department (OPD) at the UHC.

The outreach services involve community engagement through community volunteers. Population demographics and prevalence of non-communicable diseases in the community served by the ORCs are reported by the CHW. A population of nearly 12,000 is served in these communities, of which approximately 1000 of them have one or more non-communicable diseases.

Sample size and sampling technique

Based on the assumption that at least 50% of the participants have adequate knowledge and are satisfied with the care, a relative precision of 20% was utilized, and the required sample size was calculated using the formula $4\ pq/d^2$. The sample size was determined to be 100 for each setting. Hence, 100 patients with diabetes from UHC and 200 patients (50 each from different communities) from the ORC were enrolled in the study. They were selected randomly from the list of patients with diabetes seeking care at the UHC or at the ORC. There was no duplication of participants.

Participants

All adults >18 years of age with diabetes of more than one year duration who were provided care either at the UHC or at the ORC for more than a year were included in the study. Those who were acutely ill were not included in the study.

Data Sources, measurement and statistical analysis

A structured questionnaire was developed by the investigators. Face-face interviews with eligible participants were conducted by the investigators to capture the data of demographics, knowledge and satisfaction with diabetes care.

The knowledge section had a maximum score of 14 and minimum score of 0. Those whose correct answers were more than 11 (≥80%) were considered as having good knowledge, 7-11 (50-79%) as having average knowledge and less than 7 (<50%) as having poor knowledge of diabetes care.

The satisfaction with care section had 8 questions and each question contained 5 responses. Each question had a score of 0 to 4. The maximum possible score was 32 and the minimum was 0. Those scoring 24 and more (>75%) were considered to be satisfied with the care provided to them. Data was collected during the months of March-May 2022.

Data was entered using Epi-Data 3.1 software and analyzed using SPSS version 23. Descriptive statistics were calculated using proportions for categorical variables and means (SD) for continuous variables. Measures of central tendency and SD were calculated for the aggregate scores on knowledge and satisfaction with care. The association of adequate knowledge and satisfaction with care with demographic factors, duration of diabetes, number of visits, presence of complications and type of treatment was calculated using Chi-square test. *P*-value <0.05 was considered to be significant.

Institutional Review Board Clearance (IRB) and Ethics Committee (EC) Approval

This proposal was approved by the institutional review board and ethics committee-wide IRB Min.No 14502 (OBSERVE) dated 23.02.2022.

RESULTS

A total of 300 participants with diabetes duration of more than a year were contacted. All participants gave their consent. One hundred patients seeking care at the urban health center (UHC) and 200 patients from the outreach clinics (ORC) participated in the study.

The socio-demographic distribution of respondents (Table 1) was almost similar in both settings. There were a greater proportion of Hindus (p = 0.005) and those with middle school education (p = 0.04) in the ORC and more patients had diabetes of more than 10 years of duration (p = 0.02) in the UHC. The mean (SD) age of the participants at the urban health center was 55.08 (10.63) years and the median age was 55 years with a range of 28-80 years. Similarly, the mean (SD) age of the participants from the outreach clinics was 55.24 (11.49) years and the median age was 55 years with a range of 31-93 years.

The mean (SD) years of education of the participants at the UHC was 4.7 (3.88), median of 5.0 with a range of 0-17 years of education. Similarly for the ORC, the mean (SD) years of education was 5.04 (3.81), median of 5.0 with a range of 0-15 years of education. The mean (SD) duration of diabetes among the participants at the UHC was 8.8 (5.89) years, median of 8 years and range of 1-29 years. Whereas, the mean (SD) duration of diabetes among the participants at

Table 1. Distribution of respondents according to socio-demographics (N = 300)

Variable	UHC (n = 100), No (%)	ORC (n = 200), No (%)	*p*
Age in years			
<45	18 (18)	37 (18.5)	0.91
45-60	51 (51)	98 (49.0)	0.74
>60	31 (31)	65 (32.5)	0.79
Sex			
Male	18 (18)	48 (24)	0.24
Female	82 (82)	152 (76)	
Religion			
Hindu	70 (70)	168 (84)	**0.005**
Others	30 (30)	32 (16)	
Education			
No education	29 (29)	51 (25.5)	0.52
Primary	34 (34)	62 (31.0)	0.60
Middle school	15 (15)	51 (25.5)	**0.04**
High school	21 (21)	35 (17.5)	0.47
College	1 (1)	1 (0.5)	0.62
Occupation			
Unemployed	65 (65)	129 (64.5)	0.93
Employed	35 (35)	71 (35.5)	
Duration of diabetes in years			
<5	28 (28)	58 (29.0)	0.86
5-10	38 (38)	99 (49.5)	0.06
>10	34 (34)	43 (21.5)	**0.02**
Diabetes treatment			
OAD	89 (89)	173 (86.5)	0.54
Insulin	11 (11)	27 (13.5)	
Co-morbidities			
Yes	68 (68)	137 (68.5)	0.93
Frequency of clinic visits			
≤5	63 (63)	114 (57.0)	0.32
>5	37 (37)	86 (43.0)	

UHC – Urban Health Center; ORC – Outreach Clinic

the ORC was 7.57 (4.97) years, median of 6 years and range of 1-30 years.

Most of the participants had only one co-morbidity and hypertension was the most common condition in both settings. Other co-morbidities reported were dyslipidemia, mental illness, seizure disorder, rheumatoid arthritis and tuberculosis. The mean (SD) number of visits at the UHC was 5.10 (2.03) with a median of 5 and a range of 0-12 visits. For the ORC, the mean (SD) number of visits was 5.5 (1.85), median of 5 and a range of 2-11 visits.

Knowledge regarding diabetic diet and exercises was good at the UHC. Participants at the UHC had average knowledge regarding the target fasting glucose level, annual screening tests for proteinuria, eye exam, follow-up visit date and medication details. However, they had poor knowledge about the target postprandial glucose value, other annual blood tests (creatinine, HbA1c, fasting lipid profile), foot exam and foot care.

At the ORC, knowledge of diabetic diet was good. Participants had average knowledge regarding exercises, target fasting and postprandial glucose values, annual screening tests for proteinuria, foot exam, eye exam, follow-up visit and medication details. However, poor knowledge on other annual blood tests and foot care was noted.

The participants at the ORC had significantly better knowledge on target fasting and postprandial glucose values (p = 0.01, 0.03 respectively). They also had better knowledge about annual tests including creatinine, HbA1c (glycated hemoglobin) and cholesterol (p = 0.01) than the participants at the UHC as seen in Table 2.

Participants from the UHC with diabetes of more than 8 years duration [p = 0.002, OR 95% CI = 0.15 (0.04-0.58)], with co-morbidities [p = 0.02, OR 95% CI = 0.15 (0.02-0.99)] and on insulin [p = 0.003, OR 95% CI = 0.15 (0.04-0.59)] were found to have good knowledge regarding diabetes care. This was found to be statistically significant (Table 3).

On the contrary, patients from the ORC who received more than primary school education [p = 0.0001, OR 95% CI = 0.21 (0.10-0.41)] and belonging to non-Hindu religion [p = 0.001, OR 95% CI = 0.27 (0.12-0.59)] were found to have significantly higher knowledge regarding diabetes care (Table 4).

Satisfaction with care (Tables 5 and 6)

At the UHC, the patients were satisfied with the explanation given by doctors and pharmacists, the counselling performed by the nurses, and the short waiting time at the pharmacy. About half of them said they would strongly recommend this center to others. However, patients at the UHC had longer waiting times to get an appointment and to be seen by doctors. Only two-thirds of them were satisfied with the registration system.

At the ORC, waiting time to get an appointment or to see the doctor was much shorter, similar to the waiting time at the pharmacy. Patients were satisfied with the registration system, the explanation by the doctors and pharmacists and the counselling by the nurses. The majority of them said they would strongly recommend the clinic to others.

Satisfaction with care at the ORC was significantly better in terms of waiting time for appointments (p = 0.00001), doctor consultation (p = 0.00001), the registration system (0.00001), and counselling by the nurse (p = 0.01). A higher number of patients were willing to recommend diabetes care services to others (0.00001).

There was no significant difference in satisfaction with regards to the explanation by the doctors and the pharmacists and the waiting time at the pharmacy between the UHC and the ORC (Table 5).

At the UHC, patients with primary school education or with lower educational attainment were found to have higher satisfaction with care. This was statistically significant [p = 0.003, OR 95% CI = 4.15(1.59-10.84)]. There was no

Table 2. Knowledge regarding diabetes care at the UHC/ORC* (N = 300)

Knowledge questions – Do you know about	UHC (n = 100) No (%)	ORC (n = 200) No (%)	Z-score	*p*
Diabetic diet	94 (94)	194 (97)	1.25	0.21
Exercises	85 (85)	155 (77.5)	1.53	0.13
Fasting glucose target value	59 (59)	147 (73.5)	2.55	**0.01**
Postprandial glucose target value	42 (42)	111 (55.5)	2.21	**0.03**
Annual blood tests – does not know any tests	14 (14)	31 (15.5)		
Knows only one test	51 (51)	87 (43.5)		
Knows only two tests	22 (22)	31 (15.5)		
Knows all three tests (Creatinine/HbA1c/Fasting lipid profile)	13 (13)	51 (25.5)	2.49	**0.01**
Urine test for proteinuria	56 (56)	102 (51)	0.82	041
Annual foot exam	42 (42)	108 (54)	1.96	0.05
Foot care	44 (44)	81 (40.5)	0.58	0.56
Annual eye exam	66 (66)	131 (65.5)	0.09	0.93
Follow-up visit date	78 (78)	147 (73.5)	0.85	0.39
Medications taken – knows fully	63 (63)	113 (56.5)	1.08	0.28
Knows Partially	29 (29)	81 (40.5)		
Does not know at all	8 (8)	6 (3)		

UHC – Urban Health Center; ORC – Outreach Clinic

association between other factors and satisfaction with care at the UHC (Table 6).

There was no association between age, gender, religion, education, occupation, duration of diabetes, presence of co-morbidities, number of clinic visits and type of treatment with satisfaction with care at the ORC.

In both settings, only half of the participants had average knowledge on diabetes care. Majority of the participants

Table 3. Factors influencing knowledge of diabetes care at the UHC (N = 100)

Variable	Good knowledge (n = 15), No (%)	Average/Poor knowledge (n = 85), No (%)	X^2 value (*p*)	Odds ratio (95% CI)
Age in years				
≤55	7 (13.5)	45 (86.5)	0.20 (0.65)	0.78 (0.26-2.34)
>55	8 (16.7)	40 (83.3)		
Sex				
Male	3 (16.7)	15 (83.3)	**0.73 (FE)** (0.83)	1.17 (0.29-4.65)
Female	12 (14.6)	70 (85.4)		
Religion				
Hindu	9 (12.9)	61 (87.1)	**0.37 (FE)** (0.36)	0.59 (0.19-1.84)
Others	6 (20.0)	24 (80.0)		
Education				
≤Primary school	12 (19.0)	51 (81.0)	2.19 (0.14)	2.67 (0.70-10.16)
>Primary school	3 (8.1)	34 (91.9)		
Occupation				
Unemployed	11 (16.9)	54 (83.1)	0.54 (0.46)	1.58 (0.46-5.38)
Employed	4 (11.4)	31 (88.6)		
Duration of diabetes				
≤8 years	3 (5.4)	53 (94.6)	**9.28 (0.002)**	**0.15 (0.04-0.58)**
>8 years	12 (27.3)	32 (72.7)		
Co-morbidities				
No	1 (3.1)	31 (96.9)	**0.03 (FE) (0.02)**	**0.12 (0.02-0.99)**
Yes	14 (20.6)	54 (79.4)		
No. of clinic visits				
≤5 times	8 (12.7)	55 (87.3)	0.71 (0.40)	0.62 (0.21-1.89)
>5 times	7 (18.9)	30 (81.1)		
Diabetes treatment				
OAD	10 (11.2)	79 (88.8)	**0.01 (FE) (0.003)**	**0.15 (0.04-0.59)**
Insulin	5 (45.3)	6 (54.5)		

Table 4. Factors influencing knowledge of diabetes care at the ORC (N = 200)

Variable	Good knowledge (n = 52), No (%)	Average/Poor knowledge (n = 148), No (%)	X^2 value (*p*)	Odds ratio (95% CI)
Age in years				
≤55	26 (24.8)	79 (75.2)	0.18 (0.68)	0.87 (0.46-1.64)
>55	26 (27.4)	69 (72.6)		
Sex				
Male	17 (35.4)	31 (64.6)	2.91 (0.09)	1.83 (0.91-3.69)
Female	35 (23.0)	117 (77)		
Religion				
Hindu	36 (21.4)	132 (78.6)	**11.41 (0.001)**	**0.27 (0.12-0.59)**
Others	16 (50)	16 (50)		
Education				
≤Primary school	15 (13.3)	98 (86.7)	**21.87 (0.0001)**	**0.21 (0.10-0.41)**
>Primary school	37 (42.5)	50 (57.5)		
Occupation				
Unemployed	28 (21.7)	101 (78.3)	3.48 (0.06)	0.54 (0.29-1.04)
Employed	24 (33.8)	47 (66.2)		
Duration of diabetes				
≤6 years	30 (28.3)	76 (71.7)	0.62 (0.43)	1.29 (0.68-2.44)
>6 years	22 (23.4)	72 (76.6)		
Co-morbidities				
No	14 (22.2)	49 (77.8)	0.68 (0.41)	0.74 (0.37-1.50)
Yes	38 (27.7)	99 (72.3)		
No. of clinic visits				
≤5 times	31 (27.2)	83 (72.8)	0.19 (0.66)	1.16 (0.61-2.19)
>5 times	21 (24.4)	65 (75.6)		
Diabetes Treatment				
OAD	43 (24.9)	130 (75.1)	0.87 (0.35)	0.66 (0.28-1.58)
Insulin	9 (33.3)	18 (66.7)		

Table 5. Satisfaction with diabetes care at the UHC/ORC (N = 300)

Satisfaction of care	UHC (n = 100) No (%)	ORC (n = 200) No (%)	Z-score	*p*
No. of days waited for appointment				
4 to >7 days	17 (17)	2 (1)		
2-3 days	39 (39)	17 (8.5)		
1 to <1 day	44 (44)	181 (90.5)	8.77	**0.00001**
Satisfied with the registration system				
Poor	1 (1)	2 (1)		
Average	31 (31)	20 (10)		
Good/Excellent	68 (68)	178 (89)	4.46	**0.00001**
Waiting time to see the doctor				
>4 hours	31 (31)	1 (0.5)		
2-4 hours	41 (41)	21 (10.5)		
<2 hours	28 (28)	178 (89)	10.74	**0.00001**
Explanation of the treatment by doctor				
Poor	1 (1)	0		
Average	8 (8)	11 (5.5)		
Good/Excellent	91 (91)	189 (94.5)	1.15	0.25
Counselling by the nurses				
Poor	3 (3)	1 (0.5)		
Average	15 (15)	15 (7.5)		
Good/Excellent	82 (82)	184 (92)	2.58	**0.01**
Waiting time at the pharmacy				
>2 hours	0	5 (2.5)		
1-2 hours	12 (12)	22 (11)		
<1 hour	88 (88)	173 (86.5)	0.36	0.72
Explanation of medicines by pharmacist				
Poor	1 (1)	1 (0.5)		
Average	17 (17)	40 (20)		
Good/Excellent	82 (82)	159 (79.5)	0.51	0.61
Recommend to others				
May be	12 (12)	0		
If needed	35 (35)	17 (8.5)		
Strongly recommend	53 (53)	183 (91.5)	7.67	**0.00001**

Table 6. Factors influencing satisfaction with diabetes care at the UHC (N = 100)

Variable	Satisfied (n = 38) No (%)	Not Satisfied (n = 62) No (%)	X^2 value (*p*)	Odds ratio (95% CI)
Age in Years				
≤55	18 (34.6)	34 (65.4)	0.53 (0.47)	0.74(0.33-1.67)
>55	20 (41.7)	28 (58.3)		
Sex				
Male	5 (27.8)	13 (72.2)	0.97 (0.32)	0.57 (0.19-1.75)
Female	33 (40.2)	49 (59.8)		
Religion				
Hindu	23 (32.9)	47 (67.1)	2.62 (0.11)	0.49 (0.21-1.17)
Others	15 (50.0)	15 (50.0)		
Education				
≤Primary school	31 (49.2)	32 (50.8)	**9.08 (0.003)**	**4.15 (1.59-10.84)**
>Primary school	7 (18.9)	30 (81.1)		
Occupation				
Unemployed	29 (44.6)	36 (55.4)	3.45 (0.06)	2.33 (0.94-5.74)
Employed	9 (25.7)	26 (74.3)		
Duration of diabetes				
≤8 years	21(37.5)	35 (62.5)	0.01 (0.91)	0.95 (0.42-2.15)
>8 years	17 (38.6)	27 (61.4)		
Co-morbidities				
No	12 (37.5)	20 (62.5)	0.01 (0.94)	0.97 (0.41-2.31)
Yes	26 (38.2)	42 (61.8)		
No. of clinic visits				
≤5 times	23 (36.5)	40 (63.5)	**0.16 (0.69)**	**0.84 (0.37-1.94)**
>5 times	15 (40.5)	22 (59.5)		
Diabetes treatment				
OAD	31 (34.8)	58 (65.2)	**0.10 (FE) (0.06)**	**0.31 (0.08-1.13)**
Insulin	7 (63.6)	4 (36.4)		

FE: Fisher's Exact calculated where the expected cell values are less than 5

were satisfied with the care provided at the ORC than at the UHC. The knowledge on diabetes care (p = 0.03) and satisfaction with care (p = 0.00001) were statistically better at the ORC as compared to the UHC as seen in Figure 1. There is a significant difference in the mean scores of satisfaction with care (p = 0.00001) between the UHC and the ORC. There is no significant difference in the mean knowledge scores in both the settings (Figure 2).

DISCUSSION

Amidst the rising incidence of diabetes in low-and-middle-income countries, there are challenges in implementing quality diabetes care to large communities due to complex factors. Accordingly, CHW are roped in globally to improve accessibility to diabetes care.[16]

Research on the role of CHWs in the delivery of diabetes care is rapidly accumulating. A systematic review on CHW-led diabetes care concluded that more research is needed to understand the role of CHWs in disease awareness, behavioral change and health outcomes.[17] Our study evaluated the knowledge and satisfaction of diabetes care delivered through CHWs in comparison to those who accessed care for diabetes directly at the UHC in low-resource urban communities.

In our study, knowledge and satisfaction of diabetes care was significantly better in the ORCs. Both systems (UHC and ORCs) had similar results in relation to interaction with doctors, nurses and pharmacists. Physician communication was found to improve patient satisfaction in various studies, particularly in primary care practices in Saudi Arabia, Japan and Qatar.[18-20] In addition to physician communication, treatment satisfaction is reported to be associated with receiving guidance on exercise therapy and tailored pharmacological therapy.[20] The system factors of waiting time for registration and consultation with doctors and nurses are found to be better in the ORCs. This is likely to be due to the system of having an assigned team consisting of physicians, CHN, CHWs for each community and the established system of follow-up for patients.

In our system of CHN-supervised, CHW-based diabetes care that is linked to the UHC, the CHWs are the vital link to the community. CHWs engage patients through in-person, telephonic and group conversations, and home visits in the community. Besides health education and monitoring of annual screening tests, they assist patients in communicating with physicians about specific needs like footwear or change in treatment regimen and facilitate appointments with physicians and referral to the UHC. The scoping review on CHW-based diabetes care included 54 articles primarily from developed countries.[16] The roles of a CHW are captured in the triad of education, support and advocacy. Our study provides evidence on the role of CHW in improving the knowledge of diabetes care in low-resource communities in developing countries.

Our study found the duration and type of treatment for diabetes and prevalence of co-morbidities of participants at the UHC and the ORCs to be similar. The higher proportion of participants who received more than primary education and who belonged to the Hindu religion in the ORCs is representative of the communities served by the ORCs. It is interesting that the number of visits of participants to the UHC and the ORCs were equal, though ORCs run weekly in the community to improve accessibility. It may not be possible to determine the health-seeking behavior of participants as they may also seek healthcare from government or other private health care systems. A community-based study in Indonesia found that no demographic factors were significantly associated with the health-seeking behavior of patients.[21] Still, health-seeking behavior plays a vital role in the management of non-communicable diseases.

Approximately 50% of our study participants are in the age group of 45-60 years with half of them diagnosed with diabetes for more than five years. A tertiary care-based study in Gujarat found the mean age of the patient population to be more than 60 years.[22] Majority of the participants are women who are dependent on family members and neighbors to accompany them to the health center. This may partially explain their health-seeking behavior. Our literature review found evidence for the influence of gender and lower income on the knowledge of diabetes.[12] The greater proportion of women in our study probably accounts for the sub-optimal knowledge on diabetes that is likely to influence their health-seeking behavior. Similar evidence is documented on the influence of socio-demographic and socioeconomic conditions on glycemic control and health outcomes.[23]

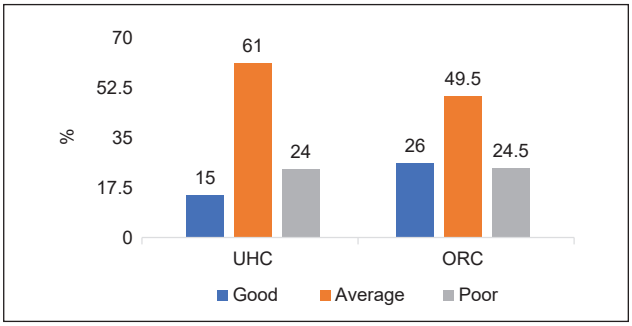

Figure 1. Knowledge of diabetes care (N = 100)/ORC (N = 200).

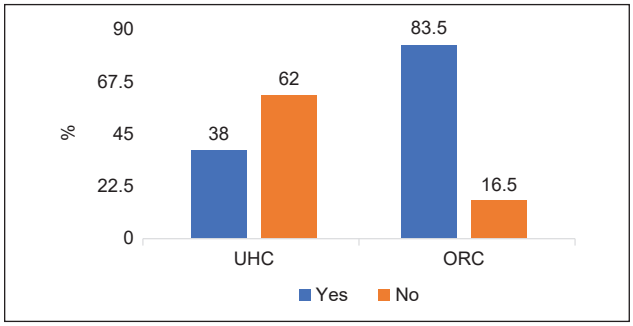

Figure 2. Satisfaction with diabetes care (N = 100)/ORC (N = 200).

The most commonly used pharmacological treatment in our study population are the oral anti-diabetes drugs (OADs). A very small proportion of our participants are on insulin. This is similar to what was reported during our audit in 2013 and similar to what is found in literature.[24,25] Most international guidelines based on studies from the West are not applicable to LMIC due to epidemiological, cultural and socio-economic variations. Diabetes is an expensive disease.[26] Often, government healthcare services provide only two to three OADs, and variable supply of insulin. Most of our patients pay out-of-pocket for treatment. There are also challenges in terms of storage facilities for insulin.

Our study identified sub-optimal knowledge of many factors related to diabetes care in the UHC and in the ORC. Better knowledge on medication and annual screening tests among participants completing middle school is identified in the ORC. This is similar to the Ethiopian study in which 43% of the study participants were identified as illiterate. Yet, more than 50% of them demonstrated good knowledge, attitude and practice towards glycemic control.[27] Better knowledge of diabetes care is found among patients with longer duration of diabetes and those on insulin in the UHC. Similar results were reported in Pakistan where university-level education and the type of therapy was associated with better knowledge of the disease.[28] The results of our study reiterate the need for health education relevant to the context of our communities.

Our results of better knowledge and satisfaction with diabetes care at the ORCs is related to the multiple factors at the ORCs that favor patient-centered care. The same team of CHW, CHN and physician assigned to each ORC facilitated better communication and continuity of care by the healthcare team. Patients within the same community foster emotional and social support in accessing the healthcare team for emergencies and screening. A systematic review on the effects of continuity of care on health outcomes of patients with diabetes found decreased mortality, complications and health service utilisation.[29] A Taiwanese study reported similar results on continuity of care for patients with diabetes.[30]

Our study in lower socio-economic communities illustrated the unique challenges faced in such settings. CHW-led diabetes care is a viable alternative to improve accessibility of services for low- resource communities. The role of CHWs and CHN should focus on patient-centered education, support to improve accessibility and advocacy for patients in the community. Patient-centred education should emphasise healthy lifestyle, self-care, health-seeking behaviour, medication adherence and treatment compliance. Health education is one of the proven scalable interventions to address the health-seeking behaviour and the social milieu of patients and to lessen the costs of diabetes care. Health education emphasising prevention and patient-centred care is likely to be one of the most cost-effective strategies in the Indian context.

CONCLUSIONS

Our study identified sub-optimal knowledge of diabetes care among study participants. In comparison, better knowledge and satisfaction with care was found at the ORC than at the UHC though no major difference was identified in terms of their socio-demographics or in the number of visits. At the UHC, better knowledge was found to be associated with longer duration of diabetes, presence of co-morbidities and use of insulin. At the ORC, better knowledge was found to be associated with receiving more than primary school education and belonging to non-Hindu religion. At the UHC, patients with lower educational attainment were found to have higher satisfaction with care. There was no association between age, gender, religion, education, occupation, duration of diabetes, presence of co-morbidities, number of clinic visits, and type of treatment with satisfaction with care at the ORC. Overall, the system factors of waiting time for registration, consultation with doctors and nurses were more favorable in the ORCs.

Strengths and Limitations

Comprehensive data collection on the factors influencing knowledge and satisfaction of diabetes care at the UHC and at the ORCs was performed.

It is a cross-sectional study. Patients who were former clients at the UHC or at the ORCs were not included in the study. Only those who were eligible based on the inclusion criteria were included. There was no duplication of participants from the UHC or ORC.

Sample size was reached by recruiting participants randomly. Participants who were included are representative of the communities in the ORCs and those who seek care at the UHC. Participants may not be representative of the general population in the society as the UHC and ORCs focus on the low-resource communities.

The UHC and the ORCs are financially supported by a private academic tertiary care hospital to provide subsidized care for the low-resource communities. The generalisbility of the results to other health care systems in India may be questionable.

As the data was collected by the co-investigators, patients may not have been forthcoming with their thoughts especially with regard to satisfaction with care. The structured questionnaire was developed locally.

The pandemic affected the accessibility of care at both centers.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
LL: Writing-review and editing; **PM:** Investigation, Data Curation, Writing-review and editing; **VK:** Investigation, Data Curation,

Writing-review and editing; **SGA:** Methodology, Writing-review and editing, Supervision; **SR:** Methodology, Resources, Writing- original draft preparation; **RAP:** Conceptualization, Methodology, Software, Formal Analysis, Resources, Writing – original draft preparation, Writing-review and editing, Supervision, Project administration.

Author Disclosure

The authors declared no conflict of interest.

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None.

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ORIGINAL ARTICLE

The Association between Betel Quid Chewing and Metabolic Syndrome Among Urban Adults in Mandalay District of Myanmar

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Abstract

Background. As the prevalence of metabolic syndrome, obesity and diabetes increase worldwide, the need to identify modifiable lifestyle risk factors also increases, especially those that may be relatively unique to a specific population. To explore a possible association between betel quid chewing and metabolic syndrome, a community-based cross-sectional study was conducted.

Methodology. Three hundred ninety-one (391) adults were interviewed and the following parameters were measured: triglycerides, HDL-cholesterol, glucose, waist circumference, body mass index and blood pressure. Multiple logistic regression was used to determine the association between betel quid chewing and metabolic syndrome while controlling for confounders.

Results. The prevalence of metabolic syndrome was similar in chewers and non-chewers, 50% and 49%, respectively. After controlling for other factors, development of metabolic syndrome was positively associated with number of betel quids chewed per day, age greater than 40 years , and a positive family history of hypertension and diabetes. Regarding the duration of betel chewing, when analyzed by sex, the risk was doubled in men compared to non-chewers (OR 2.15; 95% CI = 1.21, 3.84). As a result, a man chewing more than 10 pieces (OR 2.49; 95% CI = 1.36, 4.57) of betel quids per day for more than 10 years had a two-fold increased chance of developing the metabolic syndrome.

Conclusions. Frequency and duration of betel quid chewing may represent a behavioral lifestyle target for approaches to reduce the incidence of metabolic syndrome.

Key words: betel quid chewing, metabolic syndrome

INTRODUCTION

Worldwide, studies show that betel quid (BQ) chewing increases the risk of oral cancer and esophageal cancer.[1] In a population-based study in Taiwan where prevalence of BQ use is about 15%, data showed an increased risk of metabolic disease.[2] Arecoline, one of the betel nut alkaloids, may contribute to the metabolic abnormalities associated with BQ use. In the 3T3-L1 adipocyte, arecoline blocks insulin signaling and lipid storage in humans,[3] while in animals, it inhibits adipogenesis, induces lipolysis and interferes with insulin-induced glucose uptake.[4] The betel nut (Areca Nut) is the fourth most widely used psychoactive stimulant around the world after nicotine, alcohol and caffeine.[5] Though the actual global prevalence of this habit is yet to be documented, it has been estimated that approximately 600 million individuals worldwide chew betel quid on a daily basis.[1] The BQ is composed of areca nuts wrapped in a betel leaf coated with slaked lime. The betel leaf comes from the Piper betel vine and is a mild stimulant. In Myanmar, BQ is known as "*Kwunyar*," and is chewed with or without tobacco. The tobacco may be prepared in a variety of ways, including raw or cured (with or without drying or roasting), and other additives may include alcohol, honey, lime juice or fragrance.[6] The use of betel quid is embedded in Myanmar culture and history,[6] with an estimated prevalence of 30% to 52%; 85% of chewers add tobacco or smoked tobacco products.[7,8]

The goal of this community-based study was to explore the possible association between betel quid chewing and metabolic syndrome in a Township in Myanmar. This would allow health personnel to estimate the impact of BQ chewing on health, to raise awareness regarding the risk of these serious diseases, and to encourage preventive measures, such as legislation, to decrease smokeless tobacco use.

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METHODOLOGY

This community-based cross-sectional study evaluated adults in the Chan Aye Thar Zan Township in Mandalay District, chosen because of its heterogeneous population. The study was conducted from August 1, 2018 to April 30, 2019 through the combined efforts of the Department of Endocrinology, University of Medicine, Mandalay (UMM) and the Regional Public Health and Medical Services Department, Mandalay region, Myanmar. The study was approved by the UMM Ethical Review Committee, and written informed consent was obtained from each participant.

Participants

A minimum of 385 adult residents were needed to estimate the prevalence of betel nut chewing in Chan Aye Thar Zan Township, Myanmar given the following information: 1) The estimated prevalence of betel chewing in Myanmar Dagon (East) Township is 50%;[8] 2) Desired confidence level set at 95%; and 3) Margin of error is set at 5%. The sample size was computed using the STEPwise approach to surveillance (STEPS) Sample Size Calculator and Sampling Spreadsheet developed by the WHO.

Multi-stage sampling was conducted. First, ten quarters of the Township were randomly selected and a similar proportion of eligible households was identified in each quarter based on its population size. Next, a lottery was used to identify a single individual from each household. The staff (Health Assistants and Midwives) of the Chan Aye Thar Zan Township Medical Office contacted these potential subjects and evaluated their eligibility for participation.

Inclusion criteria were age 18 years and above, male or female of any ethnic group and willingness to participate. Exclusion criteria were pregnancy, inability to communicate or hear well and mental illness.

Data collection

Potential subjects were asked to report to the Dhamma Hall of the corresponding quarter at 0700h after a 10-hour fast. After consenting to the study, blood was obtained to measure triglycerides, HDL-cholesterol (HDL) and glucose using a Cobas 6000 analyzer. Waist circumference, height, weight and sitting blood pressure were also measured. A 15-minute face to face interview was performed using a structured questionnaire that included age, sex, occupation and details about health-harming risk factors (smoking, alcohol use, betel quid chewing and physical inactivity, as well as past medical history, drug history and family history of type 2 diabetes (T2DM), hypertension and cardiovascular disease). For betel quid users, the number of betel quid chewed per day, age at initial use, estimated years of chewing and the ingredients of preferred betel quid were recorded. At the conclusion of the interview, a sitting blood pressure was measured.

Definition of terms

Participants chewing one or more betel quids daily for one year were categorized as betel quid chewers and the remaining subjects were considered to be non-chewers.

Metabolic syndrome was defined as the presence of three out of five components using previously published consensus criteria.[9]

Central obesity (ethnicity-specific values for waist circumference for Asians): ≥90 cm in males or ≥80 cm in females

Raised triglycerides: ≥150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality

Reduced HDL cholesterol: <40 mg/dL (1.03 mmol/L) in males and <50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality

Raised blood pressure: Systolic BP ≥130 or diastolic BP ≥85 mmHg or treatment of previously diagnosed hypertension (mean value of the two measurements of sitting blood pressure was used).

Raised fasting plasma glucose: FPG ≥100 mg/dL (5.6 mmol/L), or previously diagnosed T2DM

Smoking habits were defined as: 1) Current smoker: a person who has smoked greater than 100 cigarettes or equivalents in his/her lifetime and has smoked in the last 28 days; 2) Ex-smoker: a person who has smoked greater than 100 cigarettes or equivalents in his/her lifetime but has not smoked in the last 28 days or; 3) Non-smoker: a person who has not smoked greater than 100 cigarettes or equivalents in his/her lifetime and does not currently smoke.[10]

Statistical analysis

Descriptive statistics were shown as percentages for categorical variables and mean/median for continuous variables. Differences in clinical characteristics between chewers and non-chewers were evaluated using either the chi-square test (for categorical variables) or the independent t-test (for continuous variables) (Table 1). Possible confounders were identified using simple logistic regression by determining the crude association between metabolic syndrome and the following demographic and clinical factors: age, sex, smoking, alcohol use, unhealthy food intake, sedentary lifestyle, family history of T2DM, hypertension and cardiovascular disease. Factors whose crude association with metabolic syndrome resulted in a $p>0.10$ were considered as confounders and were included in the multiple logistic regression model. Backward elimination method was then applied to determine the final model using $p\geq0.05$ as cut-off. Sex, smoking and alcohol drinking were included in the final model regardless of p-values due to strong evidence of their association with metabolic

syndrome in related literature.[11-13] Multicollinearity was checked by using the variance inflation factor (VIF) >2.5 and tolerance <0.1. The final logistic regression model was used to determine the association between betel quid chewing and metabolic syndrome. Data were analyzed with Statistics Package for Social Sciences (SPSS) version 20.

RESULTS

Of the 391 individuals who participated in the study, 182 (46.6%; 95% CI = 42%, 52%) were betel quid chewers and 209 (53.5%; 95% CI = 48%, 59%) were non-chewers, with similar proportions of men and women in each group. Most participants had completed secondary level schooling and were more than 40 years old. Chewers were more likely than non-chewers to be married, to smoke and to drink alcohol, while non-chewers were more likely to have a family history of diabetes.

The BMI (kg/m^2) of the chewers was higher than that of the non-chewers (26.7 ± 17.6 vs 23.9 ± 5.0, p<0.05) and the waist circumference (cm) of male betel quid chewers was higher than that of male non-chewers (85.7 ± 14.6 vs 81.7 ± 14.4, p<0.05) (Table 2).

Table 1. Demographic and clinical characteristics of betel quid chewers and non-chewers (n = 391). Statistically significant results are shown in bold font

Characteristics	Chewers	Non-chewers	*p*
Total participants, n (%)	182 (46.6%, CI: 0.42-0.52)	209 (53.5%, CI: 0.48-0.59)	
Male sex, n (%)	120 (65.9%)	140 (67.0%)	0.826
Female sex, n (%)	62 (34.1%)	69 (33.0%)	
Age (yrs.), mean (SD)	44.1 ± 14.1	41.27 ± 15.9	0.061
Age, n (%)			**<0.05**
<19	6 (3.3%)	26 (12.4%)	
20-29	27 (14.8%)	33 (15.8%)	
30-39	34 (18.7%)	33 (15.8%)	
40-49	43 (23.6%)	49 (23.4%)	
50-59	42 (23.1%)	37 (17.7%)	
≥60	30 (16.5%)	31 (14.8%)	
Education, n (%)			**<0.001**
Illiterate	6 (3.3%)	1 (0.4%)	
Read & write	14 (7.7%)	3 (1.4%)	
Primary school	43 (23.6%)	30 (14.4%)	
Secondary school	56 (30.8%)	53 (25.4%)	
High school	34 (18.7%)	52 (24.9%)	
University	14 (7.7%)	18 (8.6%)	
Graduate	15 (8.2%)	52 (24.9%)	
Occupation, n (%)			**<0.001**
Government servant	14 (7.6%)	23 (11.0%)	
Company employee	7 (3.8%)	36 (17.2%)	
Own business	64 (35.2%)	77 (36.8%)	
Students	5 (2.8%)	4 (1.9%)	
Unemployed	5 (2.8%)	3 (1.4%)	
Labourer	23 (12.6%)	17 (8.1%)	
Farmer	2 (1.1%)	1 (0.5%)	
Pensioner	12 (6.6%)	12 (5.7%)	
Driver	36 (19.8%)	35 (16.8%)	
Others	14 (7.7%)	1 (0.5%)	
Marital status, n (%)			**<0.001**
Married	126 (69.2%)	113 (54.0%)	
Single	38 (20.9%)	84 (40.2%)	
Divorced	4 (2.2%)	1 (0.5%)	
Widow/Widower	14 (7.7%)	11 (5.3%)	
Family history, n (%)			
Diabetes mellitus	11 (6%)	20 (11.0%)	**<0.05**
Hypertension	34 (18.7%)	44 (21.1%)	0.558
Cardiovascular disease	3 (1.6%)	8 (3.8%)	0.194
Smoking, n (%)			**<0.05**
Current smoker	46 (25.3%)	38 (18.1%)	
Ex-smoker	17 (9.3%)	11 (5.3%)	
Non-smoker	119 (65.4%)	160 (76.6%)	
Alcohol drinking, n (%)			**<0.001**
Current drinker	70 (38.4%)	41 (19.6%)	
Ex-drinker	26 (14.3%)	16 (7.7%)	
Non-drinker	86 (47.3%)	152 (72.7%)	
Exercise, n (%)	79 (43.4%)	86 (41.2%)	0.652

*NS=not significant

The metabolic syndrome was present in 131 participants (33.5%; 95% CI = 28%, 38%) and was more common in people who were more than 40 years old (80.2% vs 19.9%, $p<0.001$) (Table 3). The youngest age of a participant with metabolic syndrome was 21 years. While the proportion of women with metabolic syndrome (38.2%) was higher than those without (31.2%), it was not statistically significant ($p = 0.165$)

Overall, the prevalence of the metabolic syndrome was similar in chewers and non-chewers, 50% (95% CI = 41%, 59%) and 49% (95% CI = 41%, 58%), respectively. However,

Table 2. Values for components of the metabolic syndrome in betel quid chewers and non-chewers. Statistically significant results are shown in bold font.

Characteristics	Chewers (182, 46.6%) (mean ± SD)	Non chewers (209, 53.5%) (mean ± SD)	*p*
BMI	26.7 ± 17.6	23.9 ± 5.0	**<0.05**
Waist circumference, Male (≥90 cm)	85.7 ± 14.6	81.7 ± 14.4	**<0.05**
Female (≥80 cm)	82.4 ± 16.1	83.6 ± 11.4	0.64
Systolic blood pressure (≥130 mm Hg)	126.2 ± 21.2	129.5 ± 18.5	0.102
Diastolic blood pressure (≥85 mmHg)	81.1 ± 13.2	81.7 ± 11.6	0.769
HDL ** Male (<40 mg/dL)	49.0 ± 15.8	47.3 ± 10.2	0.288
Female (<50 mg/dL)	52.0 ± 10.9	52.9 ± 10.8	0.631
TG (≥150 mg/dL)	147.3 ± 109.6	128.9 ± 86.9	0.065
Fasting Blood glucose (≥00 mg/dL)	108.9 ± 51.6	102.7 ± 41.8	0.184

*TG – triglycerides; **HDL – High density lipoprotein cholesterol

Table 3. Factors associated with the presence or absence of metabolic syndrome. Statistically significant results are shown in bold font. Chi square test was used.

Variables	Metabolic syndrome		*p*
	Positive (131, 33.50%)	Negative (260, 66.50%)	
Duration of betel quid chewing			
Non-chewers	65 (49.62%)	144 (55.38%)	0.067
<10 years	19 (14.50%)	51 (19.62%)	
≥10 years	47 (35.88%)	65 (25.00%)	
Sex			
Male	81 (61.83%)	179 (68.85%)	0.165
Female	50 (38.17%)	81 (31.15%)	
Age			
≤40 yr.	26 (19.85%)	133 (51.15%)	**<0.001**
>40 yr.	105 (80.15%)	127 (48.85%)	
Education			
Up to Secondary school	70 (53.44%)	136 (52.31%)	0.833
More than Secondary school	61 (46.56%)	124 (47.69%)	
Occupation			0.135
Govt servant/Company employee / Own business	67 (51.15%)	154 (59.23%)	
Others (Laborer /Unemployed/ /Farmer/Driver)	60 (45.80%)	93 (35.77%)	
Students	4 (3.05%)	13 (5.00%)	
Marital status			
Married	90 (68.70%)	149 (57.31%)	**<0.05**
Single/other	41 (31.30%)	111 (42.69%)	
Smoking			
Current smoker	26 (19.85%)	58 (22.31%)	0.842
Ex-smoker	10 (7.63%)	18 (6.92%)	
Non-smoker	95 (72.52%)	184 (70.77%)	
Alcohol drinking			
Current drinker	37 (28.24%)	74 (28.46%)	0.998
Ex-drinker	14 (10.69%)	28 (10.77%)	
Non-drinker	80 (61.07%)	158 (60.77%)	
Exercise			
Doing exercise	59 (45.04%)	106 (40.77%)	0.42
Not doing exercise	72 (54.96%)	154 (59.23%)	
Family history of hypertension			
Yes	48 (36.64%)	30 (11.54%)	**<0.001**
No	83 (63.36%)	230 (88.46%)	
Family history of diabetes mellitus			
Yes	27 (20.61%)	4 (1.54%)	**<0.001**
No	104 (79.39%)	256 (98.46%)	
Family history of CVD			
Yes	5 (3.82%)	6 (2.31%)	0.394
No	126 (96.18%)	254 (97.69%)	

there was a positive association between the number of betel quids chewed each day and the presence of metabolic syndrome. There was also an increase in metabolic syndrome in those with more than 10 years of chewing. The rates of smoking, alcohol use and exercise were similar in individuals with and without metabolic syndrome. Among the participants with metabolic syndrome, most have a family history of hypertension and DM.

The betel chewers who consumed more than 10 pieces per day have 1.91 times higher risk of developing MS compared to non-betel chewers. Participants older than 40 years have 2.23 times higher risk of metabolic syndrome compared to younger individuals. Participants who are single have 39% lower risk for metabolic syndrome.

Family history of hypertension and diabetes have influence on metabolic syndrome risk (Table 4). After controlling for other variables, the predisposing factors for development of metabolic syndrome among betel chewers are number of betel quids per day, age and positive family history of hypertension and DM. Participants who chewed more than 10 pieces per day of betel quid have higher risk of metabolic syndrome compared to non-chewers (AOR 1.47; 95% CI = 1.10, 3.30). Participants who are 40 years and older have 2.23 odds of developing MS (AOR 2.23; 95%CI = 1.28, 3.92). Participants who do not have a family history of hypertension have 62% lower risk of developing MS compare to its reference group (AOR 0.38; 95% CI = 0.21,0.68). Similarly, participants who do not have family history of DM, have 90% risk reduction for MS compare to those with a family history of DM (AOR 0.10; 95% CI = 0.03, 0.32).

Age appeared to be an effect modifier in the association between betel quid chewing and metabolic syndrome. Therefore, age was used to stratify participants according to the duration of betel chewing (Table 5). Without stratification by age, a participant who chewed more than 10 pieces of betel quids per day had a 69% greater chance of developing metabolic syndrome (OR 1.69; 95% CI = 1.04, 2.76) compared to the reference group. When a cut-off threshold of 40 years was used to stratify the analysis of the number of betel quids chewed or the duration of chewing, the odds of developing metabolic syndrome changed but the 95% confidence interval crossed one.

The prevalence of metabolic syndrome was almost double in men compared to women. When all participants were analyzed, those who chewed more than 10 pieces of betel quids per day had 1.69 times higher risk for metabolic syndrome (OR 1.69; 95% CI = 1.04, 2.76) compared to the non-chewers. When sex was considered in this analysis (Table 5), the odds in men increased to 2.49 (OR 2.49; 95% CI = 1.36, 4.57). While the risk of metabolic syndrome was decreased in women (OR 0.76; 95% CI = 0.32, 1.85), this finding was not statistically significant.

Table 4. Multivariate analysis of metabolic syndrome in betel quid chewers and non-chewers

Variables	OR (CI)	AOR (CI)
No of betel quid		
Non-chewers	1	1
<10 pieces/day	0.79 (0.43-1.42)	0.71 (0.37-1.37)
≥10 pieces/day	1.69 (1.04-2.75) *	1.47 (1.10-3.30) *
Age		
≤40 yr.	1	1
>40 yr.	4.23 (2.51-7.09) **	2.23 (1.28-3.92) *
Marital status		
Married	1	1
Single/other	0.61 (0.39-0.95) *	0.81 (0.48-1.35)
Family history of hypertension		
Yes	1	1
No	0.22 (0.13-0.39) **	0.38 (0.21-0.68) *
Family history of diabetes mellitus		
Yes	1	1
No	0.06 (0.02-0.19) **	0.10 (0.03-0.32) **

**p<0.001, *p<0.05

Table 5. Association of stratified age and sex with the presence of metabolic syndrome in betel quid chewers and non-chewers. Statistically significant results are shown in bold font.

Stratified factors	Independent variables	Odd ratio (95% CI) for MS
Age	No. of betel quid pieces/day	
All age groups	Non-chewer	1
	<10	0.79 (0.43-1.43)
	≥10	**1.69 (1.04-2.76) ***
≤40 year	Non-chewer	1
	<10	0.78 (0.38-1.59)
	≥10	1.64 (0.89-3.00)
>40 year	Non-chewer	1
	<10	0.57 (0.15-2.14)
	≥10	1.42 (0.53-3.71)
Age	Years of betel quid chewing	
All age groups	Non-chewer	1
	<10	0.82 (0.45-1.51)
	≥10	1.60 (0.99-2.58)
≤40 year	Non-chewer	1
	<10	1.54 (0.67-3.56)
	≥10	1.14 (0.65-1.99)
>40 year	Non-chewer	1
	<10	0.54 (0.16-1.75)
	≥10	1.99 (0.70-5.68)
Sex	No. of betel quid pieces/day	
All subjects	Non-chewer	1
	<10	0.79 (0.43-1.42)
	≥10	**1.69 (1.04-2.76) ***
Male	Non-chewer	1
	<10	0.67 (0.29-1.54)
	≥10	**2.49 (1.36-4.57) ****
Female	Non-chewer	1
	<10	0.84 (0.35-2.06)
	≥10	0.76 (0.32-1.85)
Sex	Years of betel quid chewing	
All subjects	Non-chewer	1
	<10	0.82 (0.45-1.51)
	≥10	1.60 (0.99-2.58)
Male	Non-chewer	1
	<10	0.62 (0.23-1.62)
	≥10	**2.15 (1.21-3.84) ****
Female	Non-chewer	1
	<10	0.79 (0.34-1.82)
	≥10	0.82 (0.32-2.13)

**p<0.001, *p<0.05

Regarding the duration of betel chewing, when analyzed by sex, the risk increased in men to more than twice that of non-chewers (OR 2.15; 95% CI = 1.21, 3.84). As a result, a man chewing more than 10 pieces of betel quids per day for more than 10 years had a two-fold increased chance of developing the metabolic syndrome.

DISCUSSION

Although a variety of definitions and criteria have been used to characterize the metabolic syndrome, it remains a useful marker of a two-fold increased risk for cardiovascular disease and a five-fold increased risk of type 2 diabetes.[9] Moreover, as the prevalence of the metabolic syndrome, obesity and diabetes increases worldwide, the need to identify and mitigate risk factors increases, especially those that may be relatively unique to a specific population.

Amidst concerns that the metabolic syndrome is becoming a worldwide epidemic, a number of studies have considered whether there are modifiable lifestyle factors that might reduce this trend. A previous study in the Yangon region demonstrated significantly greater rates of components of the metabolic syndrome in urban dwellers compared to rural inhabitants.[14] In men, rates of hypercholesterolemia and hypertriglyceridemia were higher, and in both sexes, obesity (12.3% vs.7.7%; $p = 0.019$) and diabetes (17.2% vs. 9.2%; $p = 0.024$) were more common in urban residents.

Using the Modified ATP III criteria, up to 26.4% of adults in the Yangon region of Myanmar had one or more components of the metabolic syndrome: obesity (5.5%), diabetes (10.5%), overweight (16.9%), prediabetes (19.7%) and hypertension (26.4%).[15] In the Yangon study, metabolic syndrome was present in 16.6% of men and 20.5% of women. By contrast, in the current study in Mandalay, metabolic syndrome was found in 33.5% of participants, with 38.2% being men and 31.2% were women. Whether this reflects different behaviors in the two areas or a general increase in risk factors over time cannot be determined.

This study found that men who chewed betel quid more than 10 pieces per day for more than 10 years had a two-fold increased chance of developing metabolic syndrome. These data suggest that betel quid chewing is an important modifiable factor for the development of metabolic syndrome, particularly with long-term and heavy use. The risk for MS in addition to the increased risk for development of oral, pharyngeal and esophageal cancers, indicates the need for an urgent intervention at the community level to reduce BQ chewing, in the hope of significantly reducing cardiovascular and oncologic morbidity and mortality. Use of areca nut and betel quid is especially common in the entire Asia-Pacific region, including Myanmar. A 2016 Policy Review from a meeting of scientific and public health leaders representing 21 countries called for action to reduce use of this agent.[16]

Although we have shown that betel quid chewing is associated with development of metabolic syndrome, there are several limitations to this study. First, this study is cross-sectional, so causality cannot be demonstrated, and future prospective cohort studies would be necessary to validate these conclusions. Second, because self-report questionnaires were used to estimate the exposure to betel quid, misclassification may have occurred. However, using the cumulative exposure of betel quid chewing to assess the relationship to metabolic syndrome likely minimizes the residual confounding. Furthermore, we show dose–response effects between betel quid chewing and metabolic syndrome. While this reduces the possibility of a biased result, the true strength of association may be weakened.

CONCLUSION

The amount of betel quid chewed per day is associated with the development of metabolic syndrome in the Mandalay region of Myanmar. This finding provides a target for intervention at the community level. However, a number of factors may pose a challenge to any campaign to reduce betel quid use. These include the following: psychoactive effects of the areca nut, development of dependence, widespread cultural acceptance of this practice, an association with masculinity in men and a perception that the practice enhances interpersonal relationships.[17] It is our hope that identification and public awareness of these risks associated with betel quid use will lead to worldwide policies aimed at reducing its use and improving population health.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
AAA: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **SNSZ:** Methodology, Validation, Investigation, Resources, Data Curation, Writing – original draft preparation, Visualization; **AKK:** Software, Validation, Formal analysis, Investigation, Resources, Data Curation; **ACT:** Software, Validation, Investigation, Resources.

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ORIGINAL ARTICLE

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Predictors of Poor Glycemic Control and Increased Glucose Variability Among Admitted Moderate to Critical COVID-19 Patients with Type 2 Diabetes Mellitus: A Single Center Cross-sectional Study*

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Abstract

Objectives. COVID-19 exacerbates the long-standing, low-grade chronic inflammation observed in diabetes leading to heightened insulin resistance and hyperglycemia. Mortality increases with hyperglycemia and poor glycemic variability, hence, this study aims to identify the predictors associated with poor glycemic control and increased glucose variability among patients with COVID-19 and Type 2 Diabetes Mellitus (T2DM).

Methodology. A retrospective chart review of 109 patients with moderate to severe COVID-19 and T2DM admitted from March 2020 to June 2021 was done. Logistic regression was done to determine predictors for hyperglycemia and poor variability.

Results. Of the 109 patients, 78% had hyperglycemia and poor variability and 22% had no poor outcomes. Chronic kidney disease (eOR 2.83, CI [1.07-7.46], p = 0.035) was associated with increased glycemic variability. In contrast, increasing eGFR level (eOR 0.97, CI [0.96-0.99], p = 0.004) was associated with less likelihood of increased variability. Hs-CRP (eOR 1.01, CI [1.00-1.01], p = 0.011), HbA1c (eOR 1.86, CI [1.23-2.82], p = 0.003), severe COVID-19 (eOR 8.91, CI [1.77-44.94], p = 0.008) and critical COVID-19 (eOR 4.42, CI [1.65-11.75], p = 0.003) were associated with hyperglycemia. Steroid use (eOR 71.17, CI [8.53-593.54], p<0.001) showed the strongest association with hyperglycemia.

Conclusion. Potential clinical, laboratory and inflammatory profiles were identified as predictors for poor glycemic control and variability outcomes. HbA1c, hs-CRP, and COVID-19 severity are predictors of hyperglycemia. Likewise, chronic kidney disease is a predictor of increased glycemic variability.

Key words: COVID-19, type 2 diabetes, hyperglycemia, risk factors

INTRODUCTION

The Coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has had a significant impact on medicine. Among COVID-19 patients with Type 2 Diabetes Mellitus (T2DM), disease severity is increased with associated higher mortality risk.[1,2] In the TurCoviDia study, 30-day mortality was increased among COVID-19 patients with T2DM. Furthermore, older age, male gender, obesity, insulin treatment, lymphopenia and pulmonary involvement on admission were independently associated with mortality.[3] In the Coronavirus SARS-CoV-2 and Diabetes Outcomes study (CORONADO), diabetes-related phenotypes were investigated to determine their association with admitted patients with COVID-19. In the same study, body mass index (BMI), not the long-term glucose control, was independently associated with the severity of COVID-19.[4] Although the exact mechanism remains unclear, the dysregulated immune and inflammatory response of the host with COVID-19 and T2DM has been implicated.[5] Among hospitalized COVID-19 patients with T2DM, COVID-19 increases the risk of poor glycemic control.

In COVID-19, inflammatory markers such as interleukin-6 (IL-6), ferritin, D-dimer, procalcitonin, high-sensitivity C-reactive peptide (hs-CRP) and lactate dehydrogenase (LDH) are used to assess disease prognosis. However, the correlation of these inflammatory markers with poor glycemic control indices – hyperglycemia, hypoglycemia

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and glycemic variability in patients with COVID-19 and T2DM are yet to be established. Optimal glycemic variability (blood glucose within 70 to 180 mg/dL) was associated with lower mortality than poorly controlled blood glucose.[6] Since poor glycemic variability is associated with increased mortality, early intensification of treatment might be needed, hence necessitating the identification of the predictors for poor glucose control and increased glucose variability. This study aimed to identify the predictors for poor glycemic control indices and increased glucose variability among patients with moderate to critical COVID-19 infection and T2DM admitted to a tertiary hospital in Manila, Philippines. In addition, this study hypothesized that the inflammatory markers including procalcitonin, hs-CRP, LDH, D-Dimer, ferritin and IL-6 are associated with poor glycemic control and increased glucose variability.

METHODOLOGY

This is a retrospective cross-sectional study involving patients admitted at the University of Santo Tomas Hospital with a diagnosis of moderate to critical COVID-19 and T2DM from March 2020 to June 2021. Medical records of all patients who were admitted were systematically reviewed. A total of 109 patients were included in the study. A minimum of 88 patients are required for this study based on a 65.18% prevalence of patients with poorly controlled blood glucose.[6] The sample was also based on an assumed 2.5 odds ratio of any significant covariates of the outcome, poor glycemic control and increased glycemic variability. This computation also accounts for a 5% level of significance and 10% desired half-width of confidence interval.[7]

Patients who met the following criteria were included in the study: 1) ≥18 years old with moderate to critical COVID-19 infection confirmed through Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) via nasopharyngeal and/or oropharyngeal swab with T2DM admitted at the University of Santo Tomas Hospital; 2) capillary blood glucose (CBG) monitoring with at least 4-point monitoring in patients feeding per orem, 6-point monitoring timed before feeding in patients on enteral nasogastric tube feeding, 6-point monitoring timed every 4 hours in patients on parenteral nutrition or continuous nasogastric tube feeding, and 6-point monitoring timed every 4 hours in patients on nothing per orem, during 72 hours of hospital stay; and 3) availability of the following laboratory tests and inflammatory markers: complete blood count (CBC) with emphasis on absolute lymphocyte count and platelet count, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum creatinine, glycosylated hemoglobin (HbA1c), high-sensitivity C-reactive protein (hs-CRP), procalcitonin, lactate dehydrogenase (LDH), serum ferritin, interleukin-6 (IL-6) and D-dimer.

Patients who met the following criteria were excluded from the study: 1) expired before the 72nd hour of hospital stay; and 2) other types of diabetes mellitus aside from T2DM including type 1 diabetes mellitus (T1DM), gestational diabetes mellitus, monogenic diabetes syndromes and disease of the exocrine pancreas.

A total of 396 charts with a diagnosis of COVID-19 were retrieved and screened for eligibility in the study. After screening, 109 patient records passed the eligibility criteria. Data were collected and reviewed retrospectively from the medical records section of the University of Santo Tomas Hospital. Medical data were recorded using a data collection form, and each patient was assigned a numerical code. Computations for glycemic control indices and glycemic variability were derived from CBG monitoring of admitted patients during their 72 hours of hospital stay. Data analysis was handled by a biostatistician. The following were the independent variables observed in the study: 1) laboratory profile including absolute lymphocyte count, platelet count, SGOT, SGPT, serum creatinine; and HbA1c; 2) inflammatory markers including hs-CRP, LDH, IL-6, serum ferritin, procalcitonin and D-dimer. Co-variates observed were as follows: 1) demographics including age, sex, weight and body mass index using the Asia-Pacific classification; 2) comorbidities including hypertension, ischemic heart disease, cerebrovascular disease, immunodeficient state, chronic kidney disease, chronic respiratory disease, chronic liver disease, chronic heart failure, active tuberculosis, active malignancy and hematologic disease; 3) steroid-induced hyperglycemia – defined as new-onset inpatient hyperglycemia above 180 mg/dL or worsening of current glycemic control above 180 mg/dL, 24 hours after initiation of corticosteroids in patient with T2DM, 4) COVID-19 disease severity including moderate, severe and critical COVID-19 and 5) hyperglycemia inpatient therapy including the use of metformin, sulfonylurea, thiazolidinediones, sodium-glucose cotransporter-2 inhibitor (SGLT2i), dipeptidyl peptidase 4 inhibitor (DPP4i), glucagon-like peptide-1 receptor agonist (GLP1 RA), multidose basal bolus insulin therapy including the total daily dose expressed in units/kg/day, basal insulin only, bolus insulin only, premixed insulin and insulin drip.

COVID-19 disease severity was defined as 1) moderate, if the patient had signs of non-severe pneumonia (e.g., fever, cough, dyspnea, or difficulty of breathing), respiratory rate 21-30 breaths/min, SpO_2 >92% on room air is present; 2) severe, if the patient had severe pneumonia or severe acute respiratory infection (fever, cough, dyspnea, respiratory rate >30 breaths/minute, severe respiratory distress or SpO_2 ≤92% on room air); and 3) critical, if the patient presented with COVID-Acute Respiratory Distress Syndrome, sepsis or septic shock.[8]

The following are the observed dependent variables: 1) glycemic control indices[9,10] including a) glucose mean defined as the average of daily glucose value computed within 72 hours of hospital stay; b) glucose maximum, minimum and 50th percentile (median) values as derived

from the daily CBG monitoring within 72 hours of hospital stay; c) the percentage of glucose values in the target range, below and above a target value wherein a range of 70 mg/dL to 180 mg/dL was set for this study;[6] d) hypoglycemia index[9,10] which represents the average of hypoglycemic values per day (lower limit of 70 mg/dL); e) hyperglycemia index[9,10] which represents the average of hyperglycemic values (upper limit of 180 mg/dL) and 2) glycemic variability[9,10] which includes standard deviation (SD) defined as the measure of dispersion of glucose values from the mean derived from CBG values within 72 hours of hospital stay and coefficient of variation (CV) with a set threshold of 36%.

Outcome measures used in this study were the glycemic control indices and glycemic variability. Poor glycemic control indices were defined as a percentage above the target range (>180 mg/dL) of ≥25%, a percentage below the target range (<70 mg/dL) of ≥4%, or both. Cut-off values were based on the International Consensus on Time in Range.[11] A study done utilizing the data from self-monitoring blood glucose showed that "points in range" was comparable to the time in range evaluated by continuous glucose monitoring.[12] A blood glucose range of 70 – 180 mg/dL was associated with markedly lower mortality compared to individuals with poorly controlled blood glucose.[6] Increased glycemic variability was defined as a CV of ≥36%. The set threshold of 36% was used to define between stable and unstable glycemia. Lower CV was associated with lower rates of hypoglycemia.[13]

Ethical consideration

This study was carried out in accordance with the Declaration of Helsinki, Good Clinical Practice and the National Ethical Guidelines for Health and Health-Related Research 2017. This study (REC-2021-07-090-TF) was approved by the UST Hospital Research Ethics Committee.

Statistical analysis

Descriptive statistics were used to summarize the demographic and clinical characteristics of the patients. Frequency and proportion were used for categorical variables, median and interquartile range for non-normally distributed continuous variables and mean and SD for normally distributed continuous variables. Independent Sample T-test, Mann-Whitney U test and Fisher's Exact/ Chi-square test were used to determine the difference of mean, rank and frequency respectively, between patients with and without poor glycemic control and increased glucose variability. Estimated odds ratio and corresponding 95% confidence intervals from binary logistic regression were computed to determine significant predictors for poor glycemic control indices and increased glycemic variability. All statistical tests were two-tailed tests. Shapiro-Wilk test was used to test the normality of the continuous variables. Missing values were neither replaced nor estimated. Null hypotheses were rejected at 0.05 α-level of significance. STATA 13.1 (College Station, TX, USA) was used for data analysis.

RESULTS

A total of 109 patients were admitted with the diagnosis of moderate to critical COVID-19 with T2DM as a comorbidity. Among these patients, 78% developed poor glycemic control and/or increased glucose variability, while 22% had no poor outcomes. Mean age was 59 (range 47-64) years and sex distribution (male n = 62, 56.8% vs female n = 47, 43.1%) between the groups was not statistically significant. Obese body mass index (mean 28.0, range 22-34) was observed between both sexes with hypertension (n = 82, 75.2%) as the leading comorbidity. In terms of severity, 28% were classified as moderate, 19% were classified as severe and 52% were classified as critical. Steroid use was frequently observed in the poor outcome group (n = 61, 82.43%). Patients with severe and critical COVID-19 were frequently found to have poor glycemic outcomes compared to those with moderate COVID-19 (19 [22.35%], 48 [56.47%] vs 2 [8.33%], 9 [37.5%] $p = 0.009$).

In the laboratory profile of COVID-19 patients, the poor outcome group had statistically significant higher HbA1c levels (7.82 [6.96-10.28], $p = 0.002$). For the inflammatory markers, there was an increased trend in median hs-CRP in the poor outcome group compared to the other group, however, it did not reach statistical significance (116.29 [36.52-210] vs 50.38 [22.1-136.5] $p = 0.059$). The use of DPP4i and multiple basal-bolus insulin injections were more frequent in the poor outcome group. Moreover, the total daily insulin dose is higher in the poor outcome group (1 [0.6-1.3] vs 0.4 [0.1-1.1] $p = 0.034$). The clinical, demographic, laboratory, inflammatory marker and inpatient hyperglycemic therapy profiles are listed in Table 1.

In the poor outcome group, patients had higher glucose mean, glucose maximum, glucose minimum and glucose median values. Likewise, the hyperglycemia index (1.5 [0.9-2.87] vs 0.14 [0.03-0.34] $p<0.001$) and percentage above range (55 [44-73] vs 11 [10-20] $p<0.001$) was higher in the poor outcome group. Hypoglycemia was only observed in the poor outcome group, as seen in the percentage below range and hypoglycemia index. As expected, patients in the good outcome group had higher target-in-range percentages compared to the poor outcome group (100 [89.5-100] vs 46 [31-56] $p<0.001$). The glycemic control indices profile is listed in Table 2.

Hyperglycemia is frequently observed in the study population. Nonetheless, acceptable glycemic variability is observed. The patient outcomes are listed in Table 3.

COVID-19 patients on DPP4i alone were 69.71% less likely to have poor glycemic control indices. However, patients on basal-bolus insulin therapy were 10.5 times more likely to have poor glycemic control indices. For every u/kg/day increase in the patient's total daily insulin dose, the odds

Table 1. Clinical, demographic, laboratory, inflammatory markers, and inpatient hyperglycemic therapy profile among study participants (n = 109)

	Poor glycemic control and/or increased glucose variability			
	Total (n = 109)	**Yes (n = 85, 78%)**	**No (n = 24, 22%)**	***p***
	Frequency (%); Mean ± SD; Median (IQR)			
Clinical and demographic profile				
Age	59.83 ± 12.29	59.83 ± 12.15	59.79 ± 13.04	0.988
Sex				0.105
Male	62 (56.88)	52 (61.18)	10 (41.67)	
Female	47 (43.12)	33 (38.82)	14 (58.33)	
Weight, kg	75.59 ± 18.46	76.29 ± 18.19	73.26 ± 19.58	0.493
BMI, kg/m^2	28.04 ± 6.06	28.28 ± 6.03	27.21 ± 6.23	0.467
Comorbidities				
Hypertension	82 (75.23)	63 (74.12)	19 (79.17)	0.790
Chronic kidney disease	25 (22.94)	19 (22.35)	6 (25)	0.788
Ischemic heart disease	10 (9.17)	9 (10.59)	1 (4.17)	0.454
Cerebrovascular disease	10 (9.17)	7 (8.24)	3 (12.5)	0.688
Chronic respiratory disease	6 (5.5)	5 (5.88)	1 (4.17)	1.000
Chronic liver disease	3 (2.75)	3 (3.53)	0	1.000
Chronic heart failure	2 (1.83)	1 (1.18)	1 (4.17)	0.393
Immunodeficient	2 (1.83)	2 (2.35)	0	1.000
Active TB	1 (0.92)	0	1 (4.17)	0.220
Steroid use	62 (70.45)	61 (82.43)	1 (7.14)	**<0.001**
Severity of COVID-19				**0.009**
Moderate	31 (28.44)	18 (21.18)	13 (54.17)	
Severe	21 (19.27)	19 (22.35)	2 (8.33)	
Critical	57 (52.29)	48 (56.47)	9 (37.5)	
Laboratory and inflammatory markers profile				
Absolute lymphocyte Ct, cell/mm^3	1470 (1071, 1925)	1470 (1071, 1840)	1477.5 (1052, 2107)	0.611
Platelet count, 10^3/mm^3	241 (219, 291)	245 (224, 291)	226.5 (209.5, 292)	0.141
HbA1c, %	7.40 (6.79, 9.3)	7.82 (6.96, 10.28)	6.92 (6.32, 7.57)	**0.002**
SGPT, U/L	41 (28.7, 64.8)	42.1 (28.4, 65.7)	39.6 (29.1, 61.5)	0.886
SGOT, U/L	46.3 (33.2, 65.4)	46.75 (35.4, 66.2)	37.2 (26.9, 60.3)	0.255
Creatinine, mg/dL	0.94 (0.7, 1.23)	0.97 (0.72, 1.26)	0.88 (0.62, 1.16)	0.256
eGFR, ml/min/1.73m^2	80.8 (56.25, 102)	79.8 (53.6, 100.8)	87.95 (63.5, 109.5)	0.395
Procalcitonin, ng/mL	0.14 (0.7, 0.42)	0.16 (0.08, 0.44)	0.1 (0.06, 0.21)	0.102
hs-CRP, mg/L	102.12 (32.8, 185.3)	116.29 (36.52, 210)	50.38 (22.1, 136.5)	0.059
LDH, U/L	321 (244, 443)	321 (244, 464)	312.5 (229, 370)	0.215
D-Dimer, mg/L FEU	0.9 (0.5, 1.5)	0.9 (0.5, 1.6)	1.05 (0.5, 1.5)	0.662
Ferritin, ng/mL	1025 (543, 1756)	1082 (581, 2197)	721.45 (498.6, 1323)	0.215
IL-6, pg/mL	51.15 (25.38, 86.9)	62.41 (26.75, 95.5)	40.57 (24.15, 58.33)	0.142
Hyperglycemic inpatient therapy profile				
Oral Hypoglycemia agents:				
DPP4i	88 (80.73)	73 (85.88)	15 (62.5)	**0.017**
Sulfonylurea	22 (20.18)	20 (23.53)	2 (8.33)	0.150
Metformin	18 (16.51)	12 (14.12)	6 (25)	0.221
SGLT2i	7 (6.42)	5 (5.88)	2 (8.33)	0.648
Thiazolidinediones	5 (4.59)	5 (5.88)	0	0.584
Insulin Therapy:				
Basal bolus insulin therapy	61 (55.96)	58 (58.24)	3 (12.5)	**<0.001**
Insulin drip	10 (9.17)	10 (11.76)	0	0.113
Basal insulin only	9 (8.26)	5 (5.88)	4 (16.67)	0.105
Premixed Insulin	8 (7.34)	8 (9.41)	0	0.196
Bolus Only	2 (1.83)	2 (2.35)	0	1.000
Total daily insulin dose, u/kg/day	0.9 (0.5 to 1.3)	1 (0.6 to 1.3)	0.4 (0.1 to 1.1)	**0.034**

Table 2. Glycemic control profile among study participants (n = 109)

	Poor glycemic control and/or increased glucose variability			
	Total (n = 109)	**Yes (n = 85, 78%)**	**No (n = 24, 22%)**	***p***
	Median (IQR)			
Glucose mean, mg/dL	188 (153, 213)	197 (177, 230)	135.5 (126, 149.5)	**<0.001**
Glucose maximum, mg/dL	266 (231, 340)	300 (255, 353)	178.5 (157, 198.5)	**<0.001**
Glucose minimum, mg/dL	110 (90, 132)	118 (95, 137)	97.5 (83.5, 105)	**<0.001**
Glucose median, mg/dL	177 (148, 212)	190 (171, 230)	139.5 (123, 147)	**<0.001**
Percentage target in range	54 (33, 75)	46 (31, 56)	100 (89.5, 100)	**<0.001**
Percentage below range	15.5 (13.5, 36)	15.5 (13.5, 36)	-	**-**
Percentage above range	50 (36, 69)	55 (44, 73)	11 (10, 20)	**<0.001**
Hypoglycemia index	2.61 (0.02, 3.66)	2.61 (0.02, 3.66)	-	**-**
Hyperglycemia index	1.34 (0.69, 2.79)	1.5 (0.9, 2.87)	0.14 (0.03, 0.34)	**<0.001**

Table 3. Glycemic control indices and variability outcomes among study participants (n = 109)

	Frequency (%); Median (IQR)
Glycemic control indices	
Hyperglycemia; ≥25% percentage above target range	79 (72.48)
Hypoglycemia; ≥4% percentage below target range	1 (0.92)
Both hyperglycemia and hypoglycemia	2 (1.83)
Good glycemic control; >70% percentage in target range	27 (24.77)
Glycemic variability	
SD, mg/dL	50 (36, 66)
CV, %	26 (19, 34)
Glycemic variability outcome	
Poor; CV ≥36%	26 (23.85)
Good; CV <36%	83 (76.15)

of having poor glycemic control indices also increase twelvefold. For every mg/L increase in the patient's hs-CRP, the odds of having poor glycemic control indices also increased by 0.74%, and for every percent increase in the patient's HbA1c, the odds of having poor glycemic indices also increased by 86.43%. Patients on steroids were 71.2 times more likely to have poor glycemic control indices. In terms of COVID-19 severity, patients with severe COVID-19 were 8.9 times more likely to have poor glycemic control indices compared to patients with moderate COVID-19. Patients with critical COVID-19 were 4.4 times more likely to have poor glycemic control indices compared to patients with moderate COVID-19. Factors associated with poor glycemic control indices are listed in Table 4.

COVID-19 patients with chronic kidney disease were 2.8 times more likely to have increased glycemic variability. In addition, the odds of increased glycemic variability decrease by 2.11% for every ml/min/1.73m^2 increase in eGFR. For every U/L increase in SGPT, the odds of having increased glycemic variability decrease by 3.85%. Patients on thiazolidinediones were 14.9 times more likely to have increased glycemic variability. For every mg/dL increase in the patient's glucose minimum, the odds of having increased glycemic variability decrease by 4.7%. Factors associated with increased glycemic variability are listed in Table 5. The researchers failed to create a multivariate model due to low number (0 to 1) of variables left after the stepwise method from the significant variables on the univariate result.

DISCUSSION

T2DM has been described as a state of chronic low-grade inflammation and it is known that CRP, IL-1β, IL-6 and other cytokines are elevated in T2DM.[14] Upon infection with SARS-CoV-2, this preexisting chronic inflammation is further augmented leading to a heightened inflammatory response. Comorbidities including hypertension, dyslipidemia, advancing age, cardiovascular disease and obesity contribute to the ongoing inflammation which leads to hyperimmune response and increased severity of COVID-19.[2,14] Poorly controlled T2DM has been reported in several studies as a poor prognostic factor for COVID-19.[1-4] Indeed, local and international guidelines were created to address hyperglycemia in COVID-19. This retrospective study was designed to: 1) Describe the demographic characteristics, clinical and laboratory profiles, including inflammatory markers of patients with moderate to critical COVID-19 infection and T2DM; and 2) Show the correlation of the identified predictors with glycemic control and variability. This study showed that baseline HbA1c and hs-CRP are potential risk factors for hyperglycemia and poor glycemic variability. Of interest, COVID-19 severity, including severe and critical COVID-19 are predictors of hyperglycemia as well. Chronic kidney disease is likewise a predictor of poor variability. To the researcher's knowledge, this is the first study to show the association

Table 4. Factors associated with poor glycemic control among study participants (n = 109)

Parameters	Univariate		
	Estimated odds ratio	95% CI	*p*
DPP4i only	0.3029	0.1209 to 0.7586	**0.011**
Basal bolus insulin therapy	10.509	3.3132 to 33.330	**<0.001**
Total daily insulin dose, u/kg/day	12.377	1.3799 to 111.02	**0.025**
hs-CRP, mg/L	1.0074	1.0017 to 1.0130	**0.011**
HbA1c, %	1.8643	1.2340 to 2.8166	**0.003**
Steroid use	71.167	8.5331 to 593.54	**<0.001**
Severity of COVID-19			
Moderate	(reference)	-	-
Severe	8.9062	1.7654 to 44.930	**0.008**
Critical	4.4063	1.6523 to 11.750	**0.003**

Table 5. Factors associated with increased glycemic variability among study participants (n = 109)

Parameters	Univariate		
	Estimated odds ratio	95% CI	*p*
Chronic kidney disease	2.8333	1.0763 to 7.4585	**0.035**
SGPT, U/L	0.9615	0.9362 to 0.9874	**0.004**
eGFR, ml/min/1.73m^2	0.9789	0.9648 to 0.9933	**0.004**
Thiazolidinediones	14.909	1.5852 to 140.22	**0.018**
Glucose minimum, mg/dL	0.9530	0.9302 to 0.9764	**<0.001**

of clinical, laboratory, and inflammatory marker profiles to glycemic control indices and variability for patients with T2DM and COVID-19.

Among hospitalized patients, the coexistence of T2DM and COVID-19 may lead to poor blood glucose control and variability. It was observed that during the first 72 hours of admission, there was frequent hyperglycemia, low occurrence of hypoglycemia and good glycemic variability among the study population. This glycemic pattern is similar to the study of Cheng where hyperglycemia on admission was associated with disease severity in COVID-19.[2] Critical COVID-19 was frequently observed in the present study.

Patients with T2DM are in a state of low-grade chronic inflammation, and concomitant COVID-19 infection can induce high levels of cytokines including IL-6, IL1β, TNFα, MCP-1 and inducible protein-10 that confers a high degree of insulin resistance leading to hyperglycemia.[15,16] In addition, high IL-6 level, an index of hypercytokinemia, correlated with hyperglycemia and difficulties with glycemic control.[17] However, this study, did not show an association between IL-6 and hyperglycemia and glycemic variability. IL-6 is shown to be elevated both in poor and good outcome groups. Further studies with a bigger sample size should be done to further explore an association between IL-6 and hyperglycemia in COVID-19 patients.

In contrast, in a study of COVID-19 patients with T2DM, a lower incidence of elevated serum CRP has been observed among patients with well-controlled blood glucose.[6] In the same study, elevated HbA1c was observed in the poor blood glucose control group. This study showed an association between the risk of hyperglycemia and increased hs-CRP and HbA1c on admission. It may be suggested that stringent glycemic control should be observed in COVID-19 patients with elevated baseline HbA1c and hs-CRP. Other inflammatory markers including procalcitonin, LDH and D-Dimer were observed to be elevated in COVID-19 patients with T2DM.[1,2] However, these inflammatory markers were not associated with poor glycemic control and increased glycemic variability in the present study. The inflammatory markers in previous studies were used as tools for prognostication of COVID-19 severity and not as predictors for poor glycemic control and increased variability.[18,19] Further studies are needed to show the direct correlation of procalcitonin, LDH and D-dimer to glycemic control in COVID-19 patients. Although these inflammatory markers did not show an association with the studied outcomes, COVID-19 severity showed a correlation with hyperglycemia.

Laboratory profiles including absolute lymphocyte count, platelet count and SGOT did not show an association with poor glycemic control indices and increased variability. In a study by Noordam et al., the researchers analyzed the association of elevated liver enzyme concentration with glycemic variability and hyperglycemia in individuals without diabetes mellitus. In the same study, hyperglycemia is associated with elevated ALT and GGT, with the latter showing the strongest correlation. The association of AST was weaker than GGT in terms of hyperglycemia.[20] Elevated ALT, AST and GGT were not correlated with higher glycemic variability.[20] Decreased glucose disposal was probably the mechanism of hyperglycemia in patients with elevated liver enzymes.[20] In this study, patients with elevated SGPT were less likely to have poor glycemic variability, compared to the Noordam study. However, elevated SGPT and SGOT did not show an association with hyperglycemia in the present study. Noordam found that GGT is probably related to glucose metabolism as compared with SGPT and SGOT.[20] This probably explains the poor correlation of SGPT and SGOT to hyperglycemia. Further investigation is needed to prove this association.

As expected, the presence of steroid hyperglycemia showed the strongest correlation with hyperglycemia. Among the comorbidities, chronic kidney disease was associated with poor glycemic variability probably due to impaired glucose metabolism in these patients.

Elevated body mass index did not show an association with poor glycemic control indices and increased glycemic variability in the present study.

In the study population, DPP4i and basal-bolus insulin therapy were the most frequently used anti-hyperglycemic agents for COVID-19. DPP4i use was associated with improved glycemic control, however, the true correlation regarding the association with glycemic control cannot be determined. Patients who were admitted presenting with mild hyperglycemia were started with DPP4i as compared with those patients presenting with severe hyperglycemia where additional hypoglycemic agents, including insulin, were added. The causal relationship cannot be ascertained, that is whether the improved glycemic control was brought about by DPP4i use or that the baseline characteristics of the patients started on DPP4i had only modest glucose elevations resulting in improved control. Other oral hypoglycemic agents such as metformin, SGLT2i and thiazolidinediones did not show any correlation with good glucose control, possibly due to low frequency of and much later use in the study population. Of note, thiazolidinediones, specifically pioglitazone, were associated with poor glycemic variability as TZDs are known to lower glucose levels in approximately 2 weeks.[21] Pioglitazone works by activation of nuclear peroxisome proliferator-activated γ receptor hence, its glycemic effects cannot be immediately seen. Basal-bolus insulin therapy was associated with hyperglycemia, however, patients who were started on this regimen may have had severe hyperglycemia at baseline. The full therapeutic property of basal-bolus insulin therapy may not have been observed immediately during the initial 72 hours resulting in hyperglycemia. In addition, steroid-induced hyperglycemia, a possible confounder, may have affected the results.

The present study has limitations. Blood glucose monitoring was tested using a capillary blood glucose meter. To minimize changes in glucose variability, patients should be on at least 4-point CBG monitoring (e.g., three times a day before meals and at bedtime), similar to the study done by Tura et al.[9] However, continuous glucose monitoring is still recommended as more data points are required to obtain more accurate glucometrics such as percentage of target glucose in range, percentage above target range, percentage below target range, SD and CV. However, a strength of this study is that it reflects the commonly used modality of blood glucose monitoring in inpatient settings. Another limitation is that because of the study's observational nature, no causality was ascertained. Also, the researchers failed to create a multivariate model due to the low number of variables left after the stepwise approach. Increasing the sample size may improve this limitation. The researchers recommend doing prospective studies using continuous glucose monitoring to reflect the complete inter- and intraday blood glucose variations.

CONCLUSION

This study was able to identify potential predictors of poor glycemic control and increased glucose variability. Clinical predictors include chronic kidney disease for increased glycemic variability and COVID-19 severity for poor glycemic control, mainly hyperglycemia. Laboratory parameters such as HbA1c and hs-CRP were associated with poor glycemic control, mainly hyperglycemia.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
JPMB: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration, Funding acquisition; **EM:** Conceptualization, Methodology, Validation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **BM:** Conceptualization, Methodology, Validation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition.

Author Disclosure
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ORIGINAL ARTICLE

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Hypothalamic-Pituitary-Adrenal Axis Activity in SARS-CoV-2 Infected Noncritically Ill Hospitalized Patients

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Abstract

Objectives. This study determined the baseline hypothalamic-pituitary-adrenal axis hormonal levels and their associated factors in noncritically ill hospitalized patients with coronavirus disease 2019 (COVID-19).

Methodology. This cross-sectional study was carried out in 91 noncritical RT-PCR-confirmed COVID-19 patients (aged 18 to 65 years) recruited consecutively from the COVID unit of two tertiary care hospitals over a period of six months. After the screening, relevant history and physical examinations were done, and blood was drawn between 07:00 am to 09:00 am in a fasting state to measure serum cortisol and plasma adrenocorticotropic hormone (ACTH) by chemiluminescent microparticle immunoassay.

Results. Of 91 patients, 54, 26, and 11 had mild, moderate, and severe COVID-19, respectively. Median values of serum cortisol (p = 0.057) and plasma ACTH (p = 0.910) were statistically similar among the severity groups. Considering a cortisol cut-off of 276 nmol/L (<10 µg/dL), the highest percent of adrenal insufficiency was present in severe (27.3%), followed by mild (25.9%) and least in the moderate (3.8%) COVID-19 cases. Using the cortisol/ACTH ratio >15, only 6.6% had enough reserve.

Conclusions. The adrenocortical response was compromised in a significant percentage of noncritically ill hospitalized patients with COVID-19, with the highest percentage of adrenal insufficiency present in severely infected cases. The HPA axis parameters of serum cortisol, plasma ACTH and cortisol/ACTH were similar across the severity of noncritical patients with COVID-19.

Key words: hypothalamic-pituitary-adrenal axis, cortisol, ACTH, SARS-CoV-2, coronavirus disease 2019

INTRODUCTION

Cortisol and adrenocorticotrophic hormones (ACTH) are under the control of hypothalamic–pituitary–adrenal (HPA) axis that maintains the adaptive changes in metabolism, cardiovascular function, and immune-modulation.[1] Any stress can increase the secretion of cortisol hormone.[1,2] Following the severe acute respiratory syndrome coronavirus (SARS-CoV) pandemic in 2003, researchers observed that the virus may affect both the hypothalamus and pituitary glands in addition to other endocrine glands.[3,4] The genome of SARS-CoV-2 is 80% identical to that of SARS-CoV-1, and both viruses produce comparable clinical signs.[4] Angiotensin-converting enzyme 2 (ACE2) receptors are abundant in the tissues of the endocrine glands and have been identified as the domain of SARS-CoV-2 infection.[5] So, it has been proposed that SARS-CoV-2 infection might lead to hormonal problems. Specifically, the HPA axis function may be affected in two ways: (i) Directly through viral invasion and cell destruction; and (ii) Indirectly through increased cytokine production and action.[6] Furthermore, a few cases of pituitary infarction with SARS-CoV-2 infection have been reported recently.[7] The immune response and viral gene expression are responsible for the formation of viral proteins which have an impact on the HPA axis function. The adrenal glands may become temporarily or permanently dysfunctional as a result of these disease processes.[8,9] The factors that influence severity and death during the coronavirus disease 2019 (COVID-19) are still unknown.[10] This study

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was designed to determine the baseline HPA axis hormonal levels in COVID-19 patients who were acutely unwell, as well as identify any associated factors that would have an impact on that determination.

METHODOLOGY

This cross-sectional study was done in the COVID-19 unit of two tertiary care hospitals from September 2021 to February 2022 after receiving ethical approval from the Institutional Review Board of a university. Informed written consent was taken from each participant. The sample size was calculated from a similar study taking into consideration a 95% confidence interval (Z = 1.96), the prevalence of adrenal insufficiency (p = 0.643), and a 10% margin of error (d = 0.1), and applying the following formula ($n = Z^2pq/d^2$).[11] The minimum sample size was computed at 88. The study was able to include a total of 91 noncritical adult patients aged 18 to 65 years with reverse transcription-polymerase chain reaction (RT-PCR) confirmed COVID-19 within 48 hours of viral detection. Exclusion criteria were: critical COVID-19, pregnancy, known chronic disorders affecting cortisol levels e.g., known case of Addison's disease or Cushing syndrome, chronic kidney disease, chronic liver disease, malignancy or any other diseases affecting the HPA axis, receiving steroids in any form in the last 3 months, oral contraceptive pill intake in the last 3 months and serum albumin <2.0 g/dL.

History (socio-demographic characteristics and symptoms) was taken and relevant vital signs and physical examinations (height, weight, pulse rate, respiratory rate, oxygen saturation, and blood pressure) were done. Initial investigations (complete blood count, electrolytes, C-reactive protein, and D-dimer) on admission were checked and all were recorded in a semi-structured questionnaire. To measure serum cortisol and plasma ACTH, blood was drawn by venipuncture before receiving any form of steroid between 07:00 am to 09:00 am, in a fasting state, via chemiluminescent microparticle immunoassay (Siemens, USA). For measurement of ACTH, blood was transported to the laboratory on ice and centrifuged at 4°C within 15 minutes of collection.

Diagnosis and classification of COVID-19 (mild, moderate, and severe) were done according to the World Health Organization's interim guidance (WHO, 2020).[12] To identify adrenal insufficiency (AI) during acute illness, different researchers have proposed different cut-offs for serum cortisol levels (15 μg/dL and 18 μg/dL by two authorities).[13,14] As no definite cut-off is decided, we considered 276 nmol/L (~10 μg/dL) as the cut-off for this study. To calculate the cortisol/ACTH ratio, serum cortisol (nmol/L) was divided by plasma ACTH (pg/mL converted to pmol/L by multiplying with 3.67).

Data were expressed in mean ± SD or median (inter-quartile range, IQR) or frequencies (percent, %). Association between two groups was analyzed by the independent samples t-test or Mann-Whitney U test, while Kruskal-Wallis one-way ANOVA test was employed for more than two groups. For qualitative variables, Pearson's chi-square test was done. Spearman's correlation test was done to determine the correlation of cortisol and cortisol/ACTH ratio with different clinical and biochemical variables. Statistical significance was considered with p-values below 0.05. Data were analyzed using SPSS software version 22.0.

RESULTS

Among 91 noncritical hospitalized patients with COVID-19, 37 (40.7%) were males and 54 (59.3%) were female. Fifty-four (59.3%), 26 (28.6%), and 11 (12.1%) patients had mild, moderate, and severe disease respectively. Only six participants (6.6%) had a sufficient reserve, defined as a cortisol/ACTH ratio greater than 15, 62 patients (62.81%) had a ratio between 3 and 15, and 25 patients (25.3%) had a ratio below 3.

The frequency of patients with serum cortisol <100 nmol/L, 100 to <200 nmol/L, 200 to <300 nmol/L, 300-350 nmol/L, and >550 nmol/L were seven (7.7%), four (4.4%), eight (8.8%), 41 (45.1%), and 31 (34%), respectively (Figure 1). Serum cortisol (p = 0.057), plasma ACTH (p = 0.910), and cortisol/ACTH (p = 0.206) were statistically similar across the severity of noncritical patients with COVID-19 (Table 1).

Considering the cortisol cut-off of 276 nmol/L (~10 μg/dL), a total of 18 out of the 91 patients (19.78%) had AI. When grouped according to the severity of COVID-19, 25.9% (14/54) of those with mild COVID had AI, 3.8% (1/26) in the moderate group, and 27.3% (3/11) in severe cases. The adrenal reserve was found to be similar among the study groups (p = 0.054) (Figure 2). All except one of these patients had plasma ACTH within the normal range (min: 5.1 pmol/L, max: 48.20 pmol/L, upper limit of normal: 46 pmol/L).

Using a serum cortisol cut-off of 15 μg/dL (~413.85 nmol/L), 39 (42.86%) had inadequate cortisol reserve. The patient group with the highest percentage of inadequate reserve was in the mild group (27, 50%), followed by the severe group (4, 36.4%) and, lastly, in the moderate group (8, 30.8%) of COVID-19.

Table 1. Comparison of serum cortisol, plasma ACTH levels, and Cortisol/ACTH ratio with disease severity of noncritical COVID-19 patients (n = 91)

Hormones	Reference values	Mild (n = 54)	Moderate (n = 26)	Severe (n = 11)	*p*
Serum cortisol, nmol/L	138-690	417.50 (271.55-576.30)	521.50 (376.25-769.75)	514.0 (210.50-677.0)	0.057
Plasma ACTH, pg/mL	Not detectable to 46	22.60 (15.35-32.77)	24.1 (14.25-34.50)	19.90 (13.80-37.80)	0.910
Cortisol: ACTH ratio	>15	4.45 (2.92, 6.50)	4.95 (3.92, 11.10)	3.70 (2.13, 8.40)	0.206

Kruskal Wallis one-way ANOVA test was done

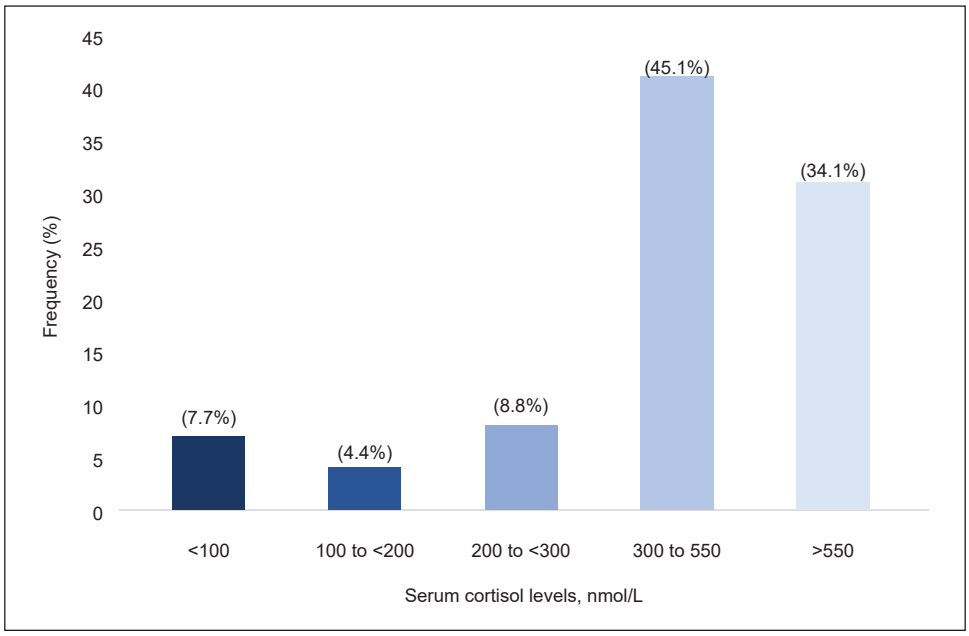
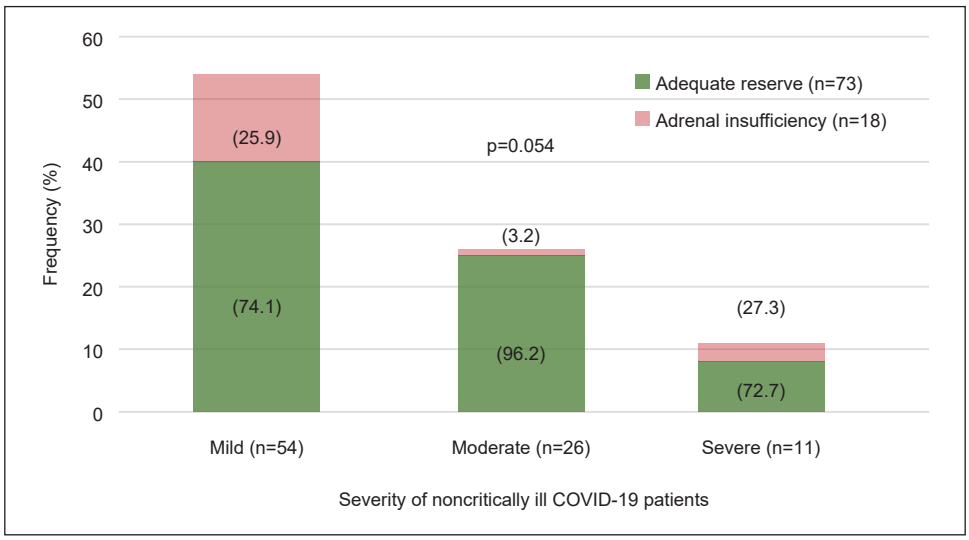


Figure 1. Stratification of the study population according to cortisol levels (n = 91).



*Cortisol reserve (cut off of 10 µg/dL = 276 nmol/L). Pearson's chi-square test was done

Figure 2. Cortisol reserve status of the study population with the severity of noncritical COVID-19 patients (n = 91).

Among all of the clinical manifestations, the prevalence of diabetes mellitus was significantly lower in patients with AI than in those with adequate cortisol reserve (11.1% vs. 47.9%, p = 0.006). The age of the participants with AI was significantly lower (40.28 ± 15.02 vs. 49.11 ± 14.52, years, p = 0.024) than those with adequate reserve. Among the different laboratory investigations, only plasma ACTH (16.59 ± 11.12 vs. 31.51 ± 23.82, pg/mL, p = 0.012) was significantly lower in patients with AI than those having adequate cortisol reserve (Table 2).

Serum cortisol had significant positive correlations with serum creatinine (r = 0.324, p = 0.018), serum ferritin (r = 0.398, p = 0.015), and plasma ACTH (r = 0.306, p = 0.003) in the study population. On the other hand, the serum cortisol/ACTH ratio had a significant negative correlation with age (r = -0.208, p = 0.048), serum creatinine (r = -0.357, p = 0.009), serum CRP (r = -0.302, p = 0.035) and serum ferritin (r = -0.528, p = 0.009) (Table 3).

DISCUSSION

The SARS-CoV-2 virus is a novel pathogen that has rapidly spread over the world since March 2020.[15] It is still unclear which factors contributed to COVID-19's severity and fatality. Unidentified primary and secondary AI may be a causative factor in the elevated fatality rates linked to COVID-19.[16] The blood cortisol cut-off in the present

Table 2. Comparison of clinical and laboratory profiles of the study population with different cortisol reserve statuses (cut-off of 10 µg/dL)

Variables	Adrenal insufficiency n = 18 (19.8%)	Adequate reserve n = 73 (80.2%)	*p*
Age, years	40.28 ± 15.02	49.11 ± 14.52	**0.024**
Sex: female	11 (61.1)	43 (58.9)	0.864
Diabetes mellitus	2 (11.1)	35 (47.9)	**0.006**
BMI, kg/m^2	22.97 ± 3.95	24.67 ± 6.31 [70]	0.282
Systolic BP, mm-Hg	124.06 ± 17.76	125.06 ± 23.58	0.874
Diastolic BP, mm-Hg	82.31 ± 10.73	79.15 ± 14.04	0.402
Neutrophil/lymphocyte ratio	3.21 (2.37-7.61) [15]	3.42 (2.17-7.91) [63]	0.712
Platelet/lymphocyte ratio	130.49 (70.34-196.01) [15]	127.83 (90.48-200.49) [63]	0.958
Na^+, mmol/L	134.0 ± 4.11 [8]	133.93 ± 6.46 [51]	0.977
S. K^+, mmol/L	4.31 ± 1.01 [8]	4.04 ± 0.81 [51]	0.401
S. ALT, U/L	51.29 ± 36.69 [7]	51.08 ± 38.14 [24]	0.990
S. Creatinine, mg/dL	0.78 ± 0.27 [8]	1.06 ± 0.47 [45]	0.107
S. CRP, mg/L	36.25 (5.53-110.55) [8]	25.0 (10.0-77.05) [41]	0.700
S. D-dimer, mg/L	0.68 (0.12-3.60) [9]	0.63 (0.21-2.81) [48]	0.895
S. Ferritin, ng/mL	274.90 (117.30-612.70) [6]	473.20 (140.0-624.40) [31]	0.615
ACTH, pg/mL	16.59 ± 11.12	31.51 ± 23.82	**0.012**

BMI (body mass index), BP (blood pressure), CRP (C-reactive protein), ACTH (adrenocorticotropic hormone)
Data were expressed in mean±SD or median (IQR) or frequency (%); [available no. in case of missing data]
Independent samples t-test or Mann-Whitney U test or Pearson's chi-square/ Fisher's exact test was done as appropriate

Table 3. Correlations of serum cortisol and cortisol/ACTH ratio with clinical and biochemical variables in the study population

Determinants of 'r'	Available no.	Cortisol		Cortisol/ACTH ratio	
		r_s	*p*	r_s	*p*
Age, years	91	0.161	0.128	-0.208	**0.048**
BMI, kg/m^2	88	-0.063	0.561	0.104	0.333
Systolic BP, mm-Hg	83	0.051	0.648	0.002	0.989
Diastolic BP, mm-Hg		-0.063	0.570	0.137	0.218
Neutrophil/lymphocyte ratio	78	0.204	0.076	0.031	0.788
Platelet/lymphocyte ratio		0.231	**0.043**	-0.012	0.916
S. Na^+, mmol/L	59	-0.083	0.531	0.176	0.181
S. K^+, mmol/L		0.028	0.831	0.082	0.539
S. ALT, U/L	31	0.228	0.218	-0.336	0.065
S. Creatinine, mg/dL	53	0.324	**0.018**	-0.357	**0.009**
S. C-reactive protein, mg/L	49	0.175	0.229	-0.302	0.035
S. D-dimer, mg/L	57	0.136	0.314	-0.009	0.948
S. Ferritin, ng/mL	37	0.398	**0.015**	-0.528	**<0.001**
P. ACTH, pg/mL	91	0.306	**0.003**	–	–

Spearman's correlation test was done

study was chosen at 276 nmol/L, whereby 18 individuals (19.78%) were diagnosed with AI. Around 25.9%, 3.8%, and 27.3% of them had mild, moderate, and severe COVID-19, respectively. There is no consensus on the diagnosis of AI in acutely ill patients and various studies used different cut-offs. An Indian cross-sectional study that used the cut-off value for blood cortisol at 138 nmol/L found that 23% of their COVID-19-affected individuals had AI and 88% had mild COVID infection.[17] If we used serum cortisol cut-off at 413.85 nmol/L, 39 (42.86%) patients in the current study showed insufficient cortisol reserve. In a comparable trial conducted in Cameroon, more than 80% of the patients exhibited an insufficient adrenal response to acute COVID-19 stress.[18] Whereas, according to Alzahrani et al., 60% of their patients had average cortisol levels below 300 nmol/L.[11]

In our study, AI was not related to the degree of severity of noncritical COVID-19 infection. Most of the studies found AI in mild cases than severely affected ones. In this study, mildly and severely affected persons with COVID-19 had nearly worse adrenal reserves than the moderately affected ones. Conventional ACTH stimulation test would have provided additional assistance in clearly recognizing AI. Kumar et al. found AI even after post-ACTH stimulation and most of their participants were mildly affected.[17]

According to one hypothesis, SARS-CoV produces an amino acid sequence that has molecular similarities with ACTH, and as a result, antibodies which developed against ACTH can inhibit the stress-induced host's cortisol response.[19] Most of our patients had plasma ACTH levels within the normal range, albeit the AI group had significantly lower levels than the normal reserve group, and showed a positive correlation with blood cortisol. This result is similar to the findings of two prior cross-sectional studies in which secondary AI was suspected.[11,18] In contrast, Gu et al., in another study, found that patients with COVID-19 had greater ACTH levels than healthy controls.[20]

The cortisol/ACTH ratio is a promising diagnostic test for primary hypoadrenalism. Lee et al. found this ratio to be safer and more convenient than the Synacthen test. They classified HPA axis status by cut-offs of 3 and 15 to define primary hypoadrenalism, secondary hypoadrenalism, and normal adrenal function respectively.[21] In the current study, more than 60% of the participants showed a cortisol/ACTH ratio between 3 and 15 indicative of secondary AI and only six persons had a ratio of more than 15. About 20% of our study participants showed baseline low serum cortisol and low normal ACTH indicating secondary AI, which is consistent with the cortisol/ACTH ratio results.

To diagnose AI, several biochemical factors are evaluated singly or in combination. Some researchers use baseline blood levels of hormones released by the adrenal cortex, whereas others use the dynamic assessment of adrenal response to typical stimuli. Among the dynamic tests, the ACTH stimulation test is commonly used. Whether the 1-μg or 250-μg ACTH stimulation test is more effective in the diagnosis of AI in non-critically ill patients is debatable.[22] We could not perform dynamic tests on any patients due to infection control precautions. However, many of our participants received steroids within a few days after being diagnosed with COVID-19, and repeat hormonal testing after recovery was difficult. Patients in the ICU were not included in our study since they had various additional confounders that may impair their adrenal function. Other limitations include the cross-sectional nature of the study design, a single measurement of ACTH, and the inability to measure cortisol binding globulin which might confound our results.

CONCLUSIONS

The absence of a significant increase in serum cortisol and low normal ACTH during acute COVID-19 infection was seen in a group of noncritically ill individuals, indicating predominant central AI. This response is not uncommon irrespective of the severity of the illness. So, the HPA axis should be evaluated on a case-to-case basis even in noncritically ill patients with COVID-19.

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Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

Credit Author Statement
HB: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Visualization; **NS:** Conceptualization, Methodology, Software, Investigation, Resources, Data Curation, Writing – review and editing, Visualization; **MSM:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation, Writing – original draft preparation, Visualization; **MAH:** Conceptualization, Methodology, Validation, Resources, Writing – review and editing, Supervision, Project administration, Funding acquisition; **ABS:** Conceptualization, Methodology, Validation, Writing – review and editing, Supervision, Project administration, Funding acquisition; **SMA:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review and editing, Supervision, Funding acquisition.

Author Disclosure
The authors declared no conflict of interest.

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ORIGINAL ARTICLE

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Association Between 25-hydroxyvitamin D Levels and Testosterone in Healthy, Non-Obese, Young Adult, Filipino Men

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Abstract

Objective. This study seeks to determine the association between vitamin D and testosterone in healthy, adult Filipino males.

Methodology. This cross-sectional study included 110 healthy, non-obese, male volunteers aged 21–40. History and physical exam were taken, and blood was drawn for vitamin D, total testosterone (TT), sex hormone binding globulin (SHBG), albumin, insulin, fasting plasma glucose, and total cholesterol. Free testosterone (FT) was calculated. Vitamin D data were classified by status and TT, FT, and SHBG levels compared using the Kruskal–Wallis test. The associations of vitamin D levels with TT, FT, and SHBG were explored using multiple regression analysis.

Results. Vitamin D levels were sufficient in 3 (2.7%), insufficient in 17 (15.45%), and deficient in 90 (81.8%) of the sample. There were no significant differences in the mean TT (p = 0.7981), FT (p = 0.8768), nor SHBG (p = 0.1838) across vitamin D status. Vitamin D was not associated with TT nor FT before or after adjustment for age and age plus body mass index (BMI). Vitamin D was associated with SHBG before and after the aforementioned adjustments, but this became insignificant on sensitivity analysis.

Conclusion. There is no association between vitamin D and TT, FT nor SHBG in our cohort with deficient vitamin D levels.

Key words: total testosterone, vitamin D, sex hormone binding globulin

INTRODUCTION

Vitamin D is known for its role in calcium-phosphorus homeostasis and bone mineralization.[1] It is a steroid hormone whose precursor, 7-dehydrocholesterol, is found in the skin and is converted by ultraviolet radiation to pre-vitamin D3 that then isomerizes to cholecalciferol and undergoes sequential hydroxylation in the liver to 25-hydroxyvitamin D (25(OH)D) and then in the kidneys to its active form, 1,25-dihydoxyvitamin D3 or calcitriol.[1] As a stable metabolite, 25(OH)D is used clinically as a biomarker of vitamin D status and the levels are classified as the following: sufficient (≥75 nmol/L or 30 ng/ml), insufficient (50-74.9 nmol/L or 20–29 ng/ml), and deficient (<50 nmol/L or 20 ng/ml).[1] Its biological actions are mediated by the vitamin D receptor (VDR), which has a ubiquitous expression in various tissues throughout the body.[1] In addition to bone health, studies have reported the involvement of hypovitaminosis D in chronic disorders such as depression, hypertension, diabetes, cardiovascular disease, autoimmune diseases, muscle dysfunction, and cancer.[2]

There is an increasing body of evidence from animal and human studies that vitamin D also modulates reproductive processes and androgen levels, specifically testosterone. While the latter plays an established role in spermatogenesis and general health of men, with physiological effects on the brain, muscle, bone, and fat mass,[3] the specific mechanisms by which vitamin D influences male reproduction remain unclear.[4] VDR and vitamin D-metabolizing enzymes are expressed in the entire reproductive male tract, including Leydig cells. This suggests an autocrine as well as paracrine action of vitamin D in the regulation of testicular function.[5]

Leydig cells express the CYP2R1 gene encoding 25-hydroxylase, much like the liver, so cellular dysfunction may influence androgen and 25(OH)D levels. VDR knockout mice also develop hypergonadotropic hypogonadism,[2]

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and human Leydig cells exposed in vitro to 1,25 (OH)D undergo modifications in steroidogenesis genes, resulting in a significant increase in testosterone biosynthesis.[2] It has also been postulated that testosterone secretion could be modulated by vitamin D-induced changes in Leydig cell calcium homeostasis involving calbindin and osteocalcin, highlighting its role in the endocrine system.[2] The negative effect of orchiectomy and testicular dysfunction on circulating 25(OH)D levels also supports their association.[6]

The association between vitamin D and reproductive hormone levels has been under investigation for more than a decade. Observational studies on the status of 25 (OH) D and circulating androgen levels are conflicting. Some did not find an association,[7-17] but others demonstrated a significant independent association of 25(OH)D with total testosterone (TT) levels after adjustment for confounder variables such as age, body mass index (BMI) and muscle mass, season, physical activity, area of residence, smoking, alcohol consumption, and presence of co-morbidities such as diabetes, hypertension, and cardiovascular disease.[18–23]

Regarding hypogonadism, an increase in 25(OH)D quartiles was associated with significantly reduced odds ratios of hypogonadism in three studies,[14,19,21] while another[21] failed to demonstrate an association. One suggested a U-shaped association (nonlinear) of 25(OH)D status and risk of hypogonadism, showing a significantly higher risk of hypogonadism in men within the highest 25(OH)D quintile compared to men in the reference quintile and a trend toward an increased risk of hypogonadism in men within the lowest 25(OH)D quintile.[9]

To date, there have been two systematic reviews and one meta-analysis to address this research question. The systematic reviews are from the same group, with their first publication in 2012,[24] updated subsequently in 2018.[25] Both papers summarized the studies but were unable to combine them into a meta-analysis. A meta-analysis from 2020[26] that included 18 studies with 9892 men with vitamin D deficiency and 10,675 controls, found a slight positive association between 25(OH)D and TT (pooled SMD: −0.23, 95% CI: −0.45 to −0.01; P = 0.04). However, heterogeneity was large (I2 = 98%, P for heterogeneity <0.00001). Subgroup analysis was performed with the studies being divided into those with community-dwelling participants and those with frail participants with a resultant decrease in I^2 (I^2 = 51%, P for heterogeneity = 0.06). A positive association was seen only in frailty states. Both the systematic review and the meta-analysis concluded that the lack of consistency between the studies may be due to different sample sizes, statistical methods, age, comorbidities, and ethnicity.

A study has identified that South Asians are more prone to vitamin D deficiency than those with European descent, presumably because of their darker skin color and excess visceral adipose tissue.[27] Another study found significant differences in 25(OH)D levels between Caucasians and Asians, suggesting a screening program for vitamin D deficiency in the latter population.[28] In this potentially high-risk group, studies on the relationship of vitamin D and testosterone are limited as well. Therefore, this study seeks to determine the association of vitamin D levels (25 (OH) D) with the level of TT, FT, and sex hormone binding globulin (SHBG) in a cohort of Filipino men, seeking to define this relationship specific to this group, most of whom are Malays. To bypass confounding from various comorbidities arising with age, we chose young, healthy, non-obese participants, similar to two previous papers,[11,12] which studied non-obese, young men with a mean age in the early twenties, less than a third of whom were smokers.

METHODOLOGY

Study design

This work is a secondary analysis of the cross-sectional study entitled "Reference Intervals of Total Testosterone in Adult Filipino Men."[29] That study determined the TT reference range among healthy young adult males. The current study received approval from both the Technical Review Board of the Department of Physiology and the Ethics Review Board of the University of the Philippines Manila. Strengthening the Reporting of Observational studies in Epidemiology (STROBE)[30] cross-sectional reporting guidelines was followed in reporting the current study findings.

Study sample

The original study included 110 healthy, Filipino young adult (aged 21-40 years) males who were studying or working at the University of the Philippines Manila between 2016 to 2019. The exclusion criteria were the following: body mass index (BMI) ≥25; hypertension (BP 140/90 mmHg); fasting plasma glucose (FPG)≥126 mg/dl; hypercholesterolemia (total cholesterol ≥240)**;** self-reported history of co-morbidities(diabetes, osteoporosis, chronic lung disease, ulcer, HIV, cancer, cerebrovascular disease, myocardial infarction, stroke, congestive heart failure, bypass, angioplasty, claudication, hyperthyroid or hypothyroid disease, infertility); or family history of hypogonadism or infertility, current use of prescription medication, history and present intake of testosterone, steroids, opioids, anticonvulsants, male fertility agents**;** smoking (present or past); alcohol consumption exceeding 600 ml ethanol(1 ml = 0.786 g) per week, and lastly, if the subject works in shifts. Data on the use of vitamin D supplements were not collected in the original study.

Data collection procedure

Participation in the original study was conditional on consent of the recruited individual. History and physical examination were performed by trained investigators following standardized procedures, as detailed in the previous manuscript. After a 10-hour fast, the blood sample was collected in plain blood collection tubes without additives (red top) within 4 hours of awakening.

Assays

FPG, albumin, and total cholesterol were analyzed using a COBAS Integra 400PLUS clinical chemistry analyzer. TT was measured using the Testosterone [I-125] RIA Kit (RK-61CT), SHBG levels with the SHBG [I-125] IRMA Kit (RK-86CT) while insulin levels were determined using the Insulin [1-125] IRMA Kit (RK-400CT). All kits mentioned were manufactured by the Institute of Isotopes Ltd., Budapest. On the other hand, 25(OH)D levels were tested using the 25-OH vitamin D total [I-125] RIA B46327 kit (Beckman Coulter, Czech Republic). The inter and intra-assay coefficients of variability (CV) for TT, SHBG, Insulin, and 25(OH)D were 12% and 8.9%, 6.04%, and 8.58%,17.1% and 4.4%, and 6.7% and 4.7%, respectively. All assays had a zero calibrator plus 5 calibrators. TT and 25(OH)D had 2 quality control test samples, while the insulin and SHBG assays had one. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), a standard measure of insulin resistance, was calculated using equation.[31]

$$\frac{\text{Fasting Insulin (microU/L) x Fasting glucose (nmol/L)}}{22.5}$$

FT was calculated using the equation developed by Vermeulen[32] using the online calculator provided by the International Society for the Study of aging Males (ISSAM).[33]

Data analysis

The data were entered into Excel and descriptive statistics were then calculated. Missing data were managed by case deletion. Data on 25(OH)D were categorized based on 25(OH)D serum status. Total testosterone, FT, and SHBG levels were compared across status using the Kruskal–Wallis test. The associations of 25(OH)D with TT and FT levels were assessed using linear regression, while the association between 25(OH)D and SHBG levels was determined using robust regression. Three models were generated for each exposure variable — Model 1 was unadjusted, Model 2 was adjusted for age, and Model 3 was adjusted for age and BMI to eliminate confounding from insulin resistance due to obesity. The adjusted regression coefficients were used to determine the strength of association between the variables of interest. Statistical significance was set at $p<0.05$. Assumptions of the linear regression analysis were tested. It was noted that the normality assumption was violated upon performing the Shapiro-Wilk test. Consequently, sensitivity analysis was performed to check the effects of outliers on the estimates derived from the regression models. Power analysis was conducted to ensure that the adjusted regression models have sufficient power given the study size. Statistical analysis was performed in R version 4.2.2.

RESULTS

All the participants (N = 110) of the original study were included in this secondary data analysis. Vitamin D levels were sufficient in three (2.7%), insufficient in 17 (15.45%), and deficient in 90 (81.8%) of study participants. Baseline characteristics like age, BMI, WHR, FPG, Insulin, and HOMA-IR were similar across vitamin D status (Table 1).

The associations of 25(OH)D with TT and FT were not significant before and after adjustment for age and both age and BMI (Table 2). The association of 25(OH)D with SHBG was significant before and after adjustment for age and both age and BMI. However, the sensitivity analysis (i.e., exclusion of outliers) resulted in non-statistically significant association between 25(OH)D and SHBG. Power analysis revealed that the adjusted regression models had at least 99.5% power when the study size and smallest effect size produced were considered.

DISCUSSION

The proportion of participants with Vitamin D deficiency in our study was high as our sample was drawn from students and office workers who are indoors from 8 AM to 5 PM. This result is similar to data from the 2013 National Nutrition Survey for Filipinos, which showed a 54% prevalence of combined vitamin D insufficiency and deficiency in the National Capital Region of the Philippines.[34] A 2015 study by Chin done in Malaysia, another Southeast Asian country,[15] showed that 23% of the participants were vitamin D deficient.

Table 1. Baseline characteristics of participants according to vitamin D status

Variable mean (SD)	Overall	Vitamin D level		*p*
		<50 nmol/L	≥50 nmol/L	
N	110	90	20	
Age (years)	27.53 (± 5.34)	27.42 (± 5.1)	28.00 (± 6.42)	0.8857
BMI[a] (kg/m^2)	22.31 (± 1.97)	22.40 (± 1.97)	21.89 (± 1.97)	0.2795
Waist-hip ratio	0.92 (± 0.05)	0.92 (± 0.05)	0.92 (± 0.05)	0.6582
Vitamin D (nmol/L)	40.91 (± 13.73)	36.19 (± 8.36)	62.12 (± 13.22)	<0.0001
TT[b] (nmol/L)	21.92 (± 10.83)	21.91 (± 11.02)	21.94 (± 10.19)	0.7981
FT[c] (nmol/L)	0.55 (± 0.33)	0.56 (± 0.33)	0.52 (± 0.31)	0.8768
SHBG[d] (nmol/L)	24.41 (± 10.63)	23.69 (± 9.77)	27.67 (± 13.73)	0.1838
FBS[e] (mg/dl)	86.36 (± 7.46)	85.91 (± 6.95)	88.35 (± 9.37)	0.2562
Insulin (mIU/ml)	10.77 (± 6.93)	10.42 (± 5.61)	12.32 (± 11.19)	0.7099
HOMA – IR[f]	1.37 (± 0.85)	1.32 (± 0.69)	1.56 (± 1.36)	0.7331

[a] Body mass index; [b] Total testosterone; [c] Free testosterone; [d] Sex hormone binding globulin; [e] Fasting blood sugar; [f] Homeostatic Model Assessment of Insulin Resistance

Table 2. The association between 25(OH)D and testosterone, free testosterone and sex hormone binding globulin

Variables	Model 1[a]		Model 2[b]		Model 3[c]	
	β Coefficient (95% CI)	*p*	β Coefficient (95% CI)	*p*	β Coefficient (95% CI)	*p*
Total testosterone	0.03 (-0.12, 0.18)	0.664	0.07 (-0.07, 0.22)	0.3160	0.05 (-0.08, 0.18)	0.469
Free testosterone	-0.00 (-0.01, 0.00)	0.662	0.00 (-0.00, 0.00)	0.9360	-0.00 (-0.00, 0.00)	0.853
Sex hormone binding globulin	0.11 (-0.01, 0.23)	0.076	0.11 (-0.01, 0.24)	0.0705	0.09 (-0.03, 0.21)	0.129

[a]Unadjusted model; [b]Adjusted for age; [c]Adjusted for age and BMI

This is the first study to evaluate the relationship between 25(OH)D levels and TT in Filipinos. There is only one similar study in the same ethnic group by Chin mentioned previously, with Chinese and Malay participants. The Malays were noted to have higher BMI and lower SHBG levels but higher TT than the Chinese. This study showed an association between 25(OH)D and TT as well as SHBG but not with FT. After adjusting for age, ethnicity, and BMI, only the direct relationship between 25(OH)D and SHBG remained significant.[15] In our study, 25(OH)D also had a direct association with SHBG even after correcting for age and BMI but not with TT nor FT. However, the association became insignificant on sensitivity analysis. The difference between this study and ours may be because majority of Filipinos are Malays,[35] while only 39% were such in this study. In addition, BMI was higher at 25 kg/m^2 in Chin's cohort while it was 22 kg/m^2 in ours. Obesity may affect both testosterone and vitamin D levels. First, insulin resistance with increasing adiposity lowers SHBG levels resulting in decreased TT levels but unchanged FT[36] and secondly, obesity has also been associated with lower 25(OH)D levels[37-39]. A 2018 meta-analysis including 55 studies showed that obesity and 25(OH)D levels may be directly related, although the result should be interpreted with caution as heterogeneity was noted to be high.[37] Possible explanations for this relationship would be deposition of vitamin D in adipose tissue[38] or volumetric dilution[39] resulting in lower circulating levels.

We also compared our results with studies with the same age group as age is also being considered as an effect modifier.[22] There are 6 other studies on participants aged less than 40 across varying ethnicities and all showed that TT was not correlated with 25(OH)D levels. These are the studies by Ramlau[7] who studied 347 Danish men aged 18–21 years; Hammoud[8] who looked at American participants with a mean age of 29; Lerchbaum[9] who examined 225 Austrian men with a median age of 35; Blomberg[10] who enrolled 1,427 Danish residents with a mean age of 34; Rudnicka[11] who included data from 198 Spanish students with a mean age of 20; and Ksiazek[12] who recruited Polish men aged 28-35. The first 2 studies had a small number of vitamin D insufficient participants, while the last four had more than 50% of their subjects with vitamin D insufficiency. A significant number of vitamin D insufficient subjects is ideal as a previous paper reported that associations between 25(OH)D and TT are stronger at the lower end of vitamin D concentrations.[20] The mean BMI of the participants in the studies ranged from 22-26 kg/m^2. Five of these studies also looked at SHBG[7-10,12] and two[7,10] showed a positive correlation with 25(OH)D. Four of these papers calculated FT[8-10,12] and one showed an inverse correlation with 25(OH)D.[10] This study had participants drawn from an infertility clinic and may not be representative of the general population. Three of these studies[7,8,10] also evaluated sperm characteristics and found that 25(OH)D levels correlated with sperm quality albeit, with conflicting results. Two studies invited healthy volunteers[7,8] in the community setting, whereas the third study recruited participants from an infertility clinic.[10] These three as well as a study by Ciccone,[18] among others, may imply that the effects of Vitamin D on reproduction are related to sperm parameters instead of hormonal levels.

The published studies with participants older than 40 years are not as concordant, as five studies[13-17] did not show an association, while six others[18-23] showed an association between 25(OH)D and TT even after correcting for confounders. We did not compare these studies with ours as the participants had varying comorbidities related to age, lifestyle, and physical activity, which may affect testosterone levels.

Our study has several strengths. The majority of the subjects had deficient 25(OH)D levels and thus, the relationship between low 25(OH)D levels and TT was well explored. Second, blood was drawn within 4 hours of awakening, thereby minimizing variability due to differing time of collection. Third, a single technician performed the assays using meticulous quality control procedures. Lastly, although the inter-assay variations of TT and insulin were above 10%, all other assays had a coefficient of variation less than 10%.

This study has several limitations. First, we tested healthy volunteers instead of drawing a sample from a population. Moreover, this being a secondary analysis, data on vitamin D supplementation and sun exposure were not available. Third, exclusion of comorbidities was through self-reporting, rather than through diagnostic testing. Fourth, we used radioimmunoassay, which has a lower accuracy and sensitivity than mass spectrometry, the gold standard. Lastly, the cross-sectional nature of the study does not allow the determination of causality.

CONCLUSION

Our study shows 25(OH)D levels are not associated with TT, FT nor SHBG in a vitamin D deficient cohort. Other possible mechanisms should be explored to explain the observed effects of vitamin D on reproductive function.

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Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
MBS: Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **RGB:** Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization; **DJTD:** Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing; **MIKC:** Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing – original draft preparation, Visualization, Supervision; **MLT:** Conceptualization, Methodology, Validation, Resources, Writing – review and editing, Supervision, Project administration, Funding acquisition.

Author Disclosure
The authors declared no conflict of interest.

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Data Availability Statement
Data is available from the corresponding author on a reasonable request.

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ORIGINAL ARTICLE

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Prevalence and Associated Factors for Thyroid Dysfunction Among Patients On Targeted Therapy for Cancers: A Single-Center Study from Thailand

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Abstract

Objective. This study aimed to explore the prevalence and associated factors of thyroid dysfunction among cancer patients treated with tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs).

Methodology. A cross-sectional study was done in patients who received TKIs at Rajavithi Hospital in 2019. For patients treated with ICI, a retrospective chart review for patients seen in 2018 to 2019 was conducted. If there were abnormal thyroid function tests (TFT), thyroid autoantibodies were tested.

Results. There were 144 patients on TKIs with a mean age of 56.0 years. Thyroid dysfunction was found in 14.6% of patients and most had subclinical hypothyroidism (n = 16, 11.1%). Imatinib (n = 11, 10.8%) and sunitinib (n = 4, 100%) were the 2 most common TKIs given to patients with thyroid dysfunction. Thyroid dysfunction was associated with male sex, chronic kidney disease and hepatitis B virus infection but not with previous thyroid disease and presence of thyroid autoantibodies.

There were 18 patients who received ICIs. The mean age was 63.3 years. Twelve patients (66.7%) used programmed cell death protein-1 antibody (anti-PD1), mainly nivolumab. Thyroid dysfunction was found in 50%, which occurred at a median duration of 46 days. Most patients had overt hypothyroidism and 55.6% needed levothyroxine replacement.

Conclusion. Thyroid dysfunctions from TKIs were mostly asymptomatic and mild in severity. Some types of TKIs might be associated with thyroid dysfunction. On the other hand, thyroid dysfunction from ICIs usually occurs within 6 months and requires levothyroxine replacement.

Key words: thyroid dysfunction, tyrosine kinase inhibitor, immune checkpoint inhibitor, immunotherapy, malignancy

BACKGROUND

Cancer incidence increased to 23.6 million cases worldwide which led to more than 10.0 million deaths in 2019.[1] Despite advances in diagnostic techniques, surgery, radiation and chemotherapy, mortality rate in some diseases such as lung cancer have not changed significantly from 40 years ago. Nowadays, our knowledge of molecular pathogenesis allows for precision medicine. Targeted therapy is a new drug strategy designed to attack abnormal cells with altered key oncogenes or tumor suppressor genes involved in tumor promotion. Selective actions affect only cancer cells with fewer side effects compared with conventional chemotherapy.[2]

Targeted anti-cancer agents are broadly classified into small-molecule inhibitors and monoclonal antibodies with tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs) as the most commonly used agents, respectively. They are reported to cause thyroid dysfunction as adverse events due to various mechanisms.

TKIs are agents that inhibit the enzyme tyrosine kinase which transfer phosphate groups on adenosine triphosphate (ATP) to the tyrosine residues of protein by phosphorylation which sends signal to regulate cell growth and differentiation.[3] There are many receptors at the cell membrane with tyrosine kinase activity e.g., epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), anaplastic lymphoma kinase (ALK), BRAF

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proto-oncogene (BRAF).[4] Increased activity of tyrosine kinase leads to uncontrolled proliferation and abnormal angiogenesis resulting in malignancies of solid organs. Over-activity of non-receptor tyrosine kinase which functions as an intracellular signal transducer, e.g., abl kinase, is responsible for pathogenesis of chronic myeloid leukemia (translocation chromosome 9, 22).[4] Indications for TKIs are non-small cell lung cancer (NSCLC), chronic myeloid leukemia (CML), renal cell carcinoma (RCC), gastrointestinal stromal tumor (GIST) and hepatocellular carcinoma (HCC).[4] TKIs available in Rajavithi Hospital, Thailand include: imatinib, sunitinib, sorafenib, gefitinib, erlotinib, afatinib, osimertinib, nilotinib, dasatinib and pazopanib. Common side effects of TKIs are gastrointestinal intolerance, anemia and folliculitis. The commonly reported TKI-related endocrinopathy is thyroid dysfunction, with a wide range of manifestations raging from asymptomatic (subclinical hypothyroid/hyperthyroid) to symptomatic patients (overt hypothyroid/ hyperthyroid). Both hypothyroidism and hyperthyroidism should be screened and treated properly to reduce complications.[5-7]

The incidence of TKIs-induced thyroid dysfunction in previous studies varied from 32-60% and depends on the type of TKI. Mechanisms of thyroid dysfunction postulated in TKI are destructive thyroiditis due to direct toxic effect as reported in sorafenib, sunitinib and axitinib.[8,9] Decreased iodine (^{123}I) scintigraphy and uptake were observed in some cases.[10] Capillary regression in thyroid gland from sunitinib,[11] increased type 3 deiodinase activity in peripheral tissue from sorafenib,[12] increased metabolism of thyroxine via potent inhibitors of CYP2C9, CYP2D6, CYP3A4/5 and increased activity of uridine diphosphate-glucuronosyl-transferase from imatinib[13] were also detected.

Immune checkpoint is the specific glycoprotein on T-cell membrane mandatory for self-recognition and controls immune response. ICI is a monoclonal antibody and binding with receptor inhibits the signal and increases anti-tumor activity. However, it can activate immune-related adverse events (irAE) such as endocrinopathies, dermatitis, colitis, hepatitis and arthritis.[14] Reported endocrinopathies are thyroid dysfunction (2.3-14.0%), hypophysitis (0.1-17.0%), insulin-dependent diabetes mellitus (0.1-0.9%) and primary adrenal insufficiency (0.5-0.9%).[15]

There are 2 pathways for immunotherapy actions. First is cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors such as ipilimumab or tremelimumab which disinhibit interleukin 2 (IL-2) production and promote cancer killing. The other drug group is involved with the programmed cell death protein-1 (PD1) pathway, namely: antibody to PD1 on T-cells (e.g., nivolumab, pembrolizumab, cemiplimab) and antibody attack PD1 ligand (anti-PD1L) on tumor cell (e.g., atezolizumab, durvalumab or avelumab) resulting in increased T-cell activity and proliferation to attack cancer cells. Indications for immunotherapy are late stage or metastatic NSCLC, RCC, malignant melanoma and other cancers from many clinical trials.[16,17]

The American Society of Clinical Oncology (ASCO) recommends monitoring of clinical symptoms and thyroid function tests (TFT) every 4-6 weeks while on immunotherapy and withdrawal of the offending drugs if grade 3 or 4 toxicity occurs. Treatment with levothyroxine for hypothyroidism or with beta blockers for thyrotoxicosis is started if persistent abnormality in TFTs is observed. A consult with an endocrinologist is likewise recommended.[18] In Thailand, there is currently no research about thyroid dysfunction associated with TKIs or ICIs use.

The objective of this study is to estimate the prevalence and associated factors for thyroid dysfunction in cancer patients who were treated with TKIs or ICI in Thailand. This will help in coming up with recommendations for best clinical care.

METHODOLOGY

This study has 2 parts: a cross-sectional study gathering data from patients who were prescribed TKIs from January to December 2019 and a retrospective chart review of cancer patients who were treated with ICIs from January 2018 to December 2019 at the hematology and oncology clinic of Rajavithi Hospital, Thailand. Baseline characteristics, history of neck surgery or radiation, underlying diseases, TKI types and duration were collected from patients and medical records. Free triiodothyronine (FT3), free thyroxine (FT4) and thyroid-stimulating hormone (TSH) were measured at recruitment. Analyses were done using Cobas e602 analyzer (Roche). The normal value for FT3 was 2.00-4.40 pg/ml (CV 1.73%), FT4 was 0.93-1.70 ng/dl (CV 2.10%) and TSH was 0.27-4.20 μIU/ml (CV 1.73%). If there were abnormal TFTs, thyroid autoantibodies which included anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase (anti-TPO) antibodies were tested. Total enumeration was performed.

Informed consent was secured. This study was approved by Rajavithi Hospital Ethics Committee (number 62036).

Inclusion criteria

Patients more than 18 years of age treated with TKIs or ICIs who were willing to participate and have TFTs were included. Patients with previous thyroid disorders were not excluded.

Exclusion criteria

Patients who had other conditions that can alter TFTs were excluded such as: acute severe medical illness requiring admission (e.g., acute myocardial infarction, acute stroke, sepsis which can alter thyroid function tests and lead to sick euthyroid state), received other drugs known to affect hormone metabolism or interfere with assay measurement (e.g., amiodarone, lithium, ethionamide, sulfonamide, iodide, gonadotropin-releasing hormone agonist (leuprorelin), interleukin-2, granulocyte/ macrophage colony-stimulating factors and biotin). Finally,

patients who developed conditions which warrant a stop or withdrawal of TKIs or ICIs for more than 1 month before assessing TFT were excluded.

Outcome

The endpoint of this study is thyroid dysfunction defined as any abnormality in the levels of FT3, FT4 or TSH. Patients with thyroid dysfunction may be asymptomatic with abnormal TFTs such as in the case of subclinical hypothyroidism (high TSH with normal FT3 and FT4) or subclinical hyperthyroidism (low TSH but normal FT4 and FT3).

Sample size calculation

From literature review, we calculated the thyroid dysfunction from TKIs from the population proportion equation.[19] The incidence of thyroid dysfunction from TKI[20]= 34.0% at 95% statistical significance (α = 0.05) and type 2 error 20% was calculated. The computed sample size is 144 patients with 13 patients drop out, so recruitment of TKIs users of at least 157 patients is required.

For logistic regression, multivariable analyses in a previous study found 3 risk factors.[20] Calculation for sample size use G*power[21] found sample size are 237 patients with at 95% statistical significance (α = 0.05), type 2 error 20%, expected odds ratio 1.5, Pr(Y=1/X=1) = 0.34, R2 other X= 0.04 (low association).

Statistical analysis

The data was analyzed using IBM SPSS Modeler 16.0. Pearson Chi-square and Fisher exact test were applied to evaluate correlation of categorical variables. Normality test with Shapiro-Wilk test was done. Paired t-test was applied for normal distributed data. Mann-Whitney U test was used for non-normal distribution continuous data comparison. Multivariable analysis was performed by logistic regression. There were no missing data in this study.

For ICIs, data was descriptive analysis via SPSS using percentage and frequency in categorical data. In continuous data, mean with standard deviation (SD) in normal distribution and median with lowest-highest data in non-normal distribution were used.

The significance value is considered as $p<0.05$

RESULTS

Tyrosine kinase inhibitors

A total of 144 patients who received TKIs were included in this study. Baseline characteristics are as shown in Table 1. There were 73 males and 71 females and the mean age ± SD was 56.0 ± 15.6 years. All patients had no exposure to drugs that may affect TFT, except for 1 post-liver transplant patient who was given cyclosporine that may cause the rare occurrence of autoimmune thyroiditis. One patient had head and neck irradiation. Three had previous thyroid disease: non-functioning thyroid nodule (n = 1), hypothyroidism of unknown cause (n = 1) and Hashimoto's thyroiditis (n = 1). Levothyroxine was given to 2 of these patients from another hospital. Baseline TFTs were available before TKIs in 2 patients which were interpreted as euthyroid (n = 1) and sick euthyroid (n = 1). Thyroid antibodies were not done except in 1 case with previously diagnosed Hashimoto's thyroiditis. Among patients with solid malignancies, 89.5% had advanced stage (Stage 4). Among patients with CML, all had chronic phase except 1 patient with blastic phase that turned to chronic phase after chemotherapy. No patient with thyroid cancer received TKIs in our study.

Most patients (83.3%) used only 1 TKI. Eighty-eight percent received the standard dose of TKI (n = 127 patients (88.2%)) while below standard and above standard doses were given in 15 and 5 patients, respectively. Frequently prescribed TKIs were: imatinib, gefitinib and nilotinib. The median duration of treatment for all TKIs was 689.5 days or 1.89 years (range: 27 to 5642 days).

Thyroid dysfunctions were found in 21 (14.6%) patients as shown in Figure 1. The most common dysfunction was subclinical hypothyroidism (n = 16, 11.1%) with 4 patients having TSH >10 mIU/L. Overt hypothyroidism was seen in 4 patients with 2 of them diagnosed after TKI use (1.4%). Subclinical hyperthyroidism was found in 3 (2.1%) patients, 1 of them had TSH <0.01 mIU/ml. Symptoms of thyroid dysfunction were presented in 2 patients with overt hypothyroidism. Treatment with levothyroxine was initiated in 4 patients. Thyroid autoantibodies were positive in 4 (19.0%) of 21 patients: overt hypothyroidism (n = 2), subclinical hyperthyroidism (n = 1), subclinical hypothyroidism (n = 1). There was no correlation between the level of antibodies and the level of abnormal TFT. One patient with a history of head and neck irradiation did not have thyroid dysfunction.

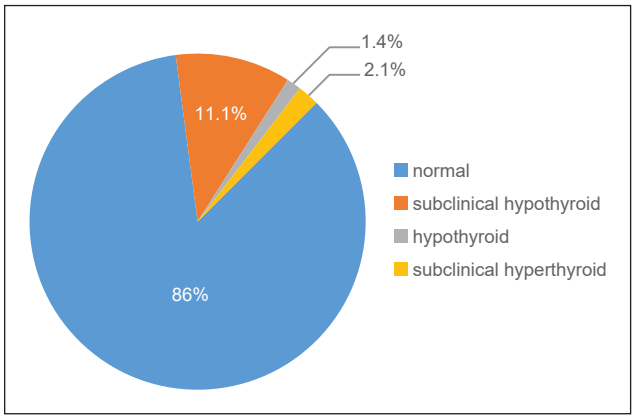

Figure 1. Type of thyroid dysfunction in TKI patients. Of the 144 users, 21 (14.6%) had thyroid dysfunction which were 16(11.1%) subclinical hypothyroid, 3 (2.1%) subclinical hyperthyroid and 2 (1.4%) hypothyroid.

The starting dose of TKIs were continued in all cases, except for 2 patients who had disease progression. Number and duration of TKI use were not significantly associated with TFT abnormality. All 4 sunitinib patients had abnormalities in TFTs including overt or subclinical hypothyroidism and treatment was indicated in 3 of these patients (Figure 2).

Male sex, comorbidity, chronic kidney disease (CKD) and hepatitis B virus (HBV) infection were associated with thyroid dysfunction. Adjusted odds ratio was still significant in male sex, CKD and HBV infection as in Table 2.

Immune checkpoint inhibitors

ICIs were given in 18 patients including 12 (66.7%) males and 6 (33.3%) females. Mean age ± SD was 63.3 ± 12.5 years old. Most patients were in the central region (n = 16, 89.0%). Five (27.8%) patients had at least 1 comorbidity which were: hypertension (n = 2), diabetes mellitus (n = 2), cerebrovascular disease (n = 1), coronary artery disease (n = 1) and cirrhosis (n = 2). Previous use of TKIs was noted in 6 patients.

The indications for ICIs were NSCLC (n = 9, 50%), RCC

Table 1. Baseline characteristics of patients who treated with TKIs

Demographic data	All (n = 144)	TFT abnormal (n = 21)	TFT normal (n = 123)	*p*
Age (mean ± SD), years	56.0 ± 15.6	58.5 ±17.2	55.6 ± 15.4	0.438
Sex (n,%)				0.040
Male	73	15, 71.4%	58, 47.2%	
Female	71	6, 28.6%	65, 52.8%	
Domicile region (n,%)				0.283
Central	118	18, 85.7%	100, 81.3%	
West	12	0, 0.0%	12, 9.7%	
Northeast	6	2, 9.5%	4, 3.3%	
East	4	0 ,0.0%	4, 3.3%	
South	3	1, 4.8%	2, 1.6%	
North	1	0, 0.0%	1, 0.8%%	
Diagnosis (n,%)				<0.001
Chronic myeloid leukemia (CML)	68	10, 47.6%	58, 47.2%	
Non-small cell lung cancer (NSCLC)	36	5, 23.8%	31, 25.2%	
Gastrointestinal stromal tumor (GIST)	35	1, 4.8%	34, 27.6%	
Renal cell carcinoma (RCC)	3	3, 14.3%	0, 0.0%	
Hepatocellular carcinoma (HCC)	2	2, 9.5%	0, 0.0%	
Staging solid tumor (n,%)	76	11	65	0.471
3	8	0, 0.0%	8,12.3%	
4	68	11, 100.0%	57, 87.7%	
Comorbid disease (n,%)				0.148
No	69	7, 33.3%	62, 50.4%	
Yes	75	14, 66.7%	61, 49.6%	
Hypertension	34	4, 19.0%	30, 24.4%	0.783
Dyslipidemia	28	3, 14.3%	25,20.3%	0.766
Diabetes mellitus	23	4, 19.0%	19, 15.4%	0.747
Chronic kidney disease	14	5, 23.8%	9, 7.3%	0.018
Coronary artery disease	4	0, 0.0%	4, 3.3%	0.528
HBV infection	4	2, 9.5%	2, 1.6%	0.102
Cerebrovascular disease	3	0, 0.0%	3, 2.4 %	1.000
Hemoglobinopathy	2	1, 4.8%	1, 0.8%	0.271
Asthma	2	1, 4.8%	1, 0.8%	0.271
HIV infection	1	0, 0.0%	1, 0.8%	1.000
Post-liver transplantation	1	1, 4.8%	0, 0.0%	0.146
Concurrent inactive cancer	3	0, 0.0%	3, 2.4%	1.000
Previous thyroid disease (n, %)	3	0, 0.0%	3, 100.0%	1.000
Thyroid nodule	1	0, 0.0%	1, 33.3%	
Hypothyroid	1	0, 0.0%	1, 33.3%	
Hashimoto's thyroiditis	1	0, 0.0%	1, 33.3%	
Number of TKI use (n, %)				0.210
1	121	16,76.2%	105, 85.4%	
2	21	4, 19.0%	17, 13.8%	
3	2	1, 4.8%	1, 0.8%	
Types of TKI use (n, %)				
Imatinib	102	11, 52.4%	91, 74.0%	0.044
Gefitinib	18	2, 9.5%	16, 13.0%	1.000
Nilotinib	16	3, 14.3%	13, 10.6%	0.705
Erlotinib	14	3, 14.3%	11, 8.9%	0.431
Sunitinib	4	4, 19.0%	0, 0.0%	0.000
Sorafenib	1	1, 4.8%	0, 0.0%	0.146
Dasatinib	6	1, 4.8%	5,4.1%	1.000
Osimertinib	2	0, 0.0%	2, 1.6%	1.000
Afatinib	4	0, 0.0%	4, 3.3%	1.000
Pazopanib	1	1, 4.8%	0, 0.0%	0.146
Duration of TKI (mean ± SD days)	1253 ± 1314	1215 ± 1332	1260 ± 1316	0.884
Dose of TKI				0.193
Standard dose	127	17, 81.0%	110, 89.4%	
Below standard dose	13	4, 19.0 %	9, 7.3%	
Above standard dose	4	0, 0.0%	4, 3.3%	

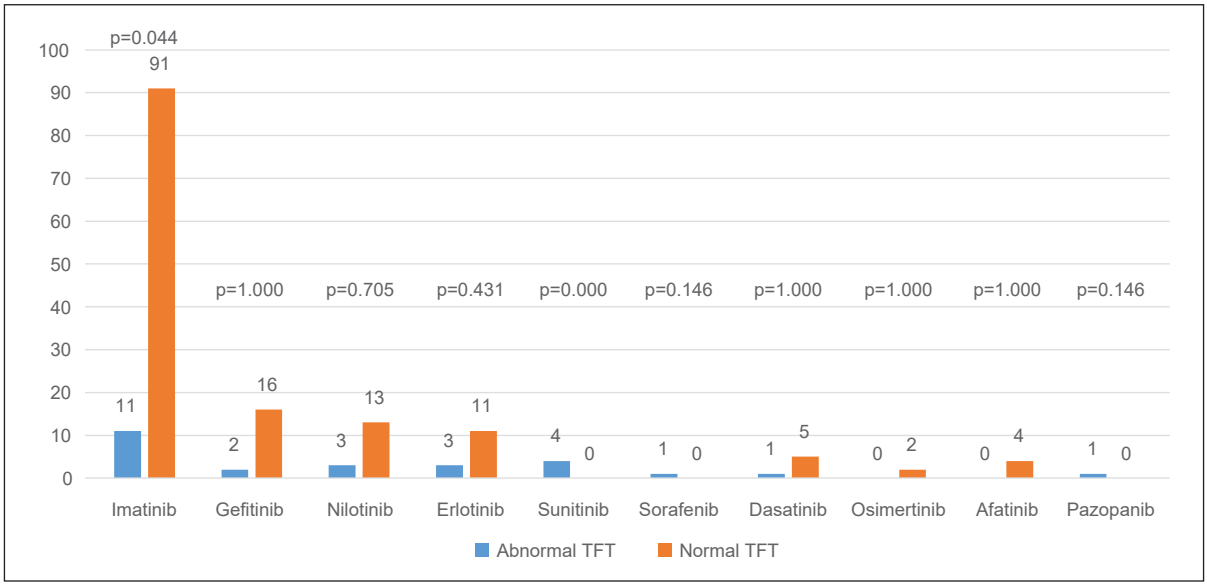

Figure 2. Thyroid dysfunctions by type of TKI.

Table 2. Risk factors associated with thyroid dysfunction from tyrosine kinase inhibitors

Variable	Crude OR (95%CI)	*p*	Adjusted OR (95%CI)	*p*
Male sex	2.802 (1.02-7.70)	0.046	3.333 (1.135-9.787)	0.028
Comorbidity	2.033 (0.768-5.382)	0.153	1.222 (0.395-3.777)	0.728
CKD	3.958 (1.178-13.301)	0.026	4.855 (1.177-20.023)	0.029
HBV infection	6.368 (0.846-47.948)	0.072	8.413 (0.917-77.187)	0.060

(n = 4, 22.2%, HCC (n = 2, 11%), malignant melanoma (n = 1, 5.6%), esophageal cancer (n = 1. 5.6%) and cecal cancer (n = 1, 5.6%). Most patients (n = 16, 88.9%) used a single drug. A combination of 2 ICIs was used in 2 patients and combination ICI with chemotherapy in 1 patient. Types of ICIs used were: nivolumab (n = 6, 33.3%), pembrolizumab (n = 4, 22.2%), durvalumab (n = 4, 22.2%), cemiplimab (n = 3, 16.7%), atezolizumab (n = 2, 11.1%) and ipilimumab (n = 1, 5.6%) as presented in Table 3.

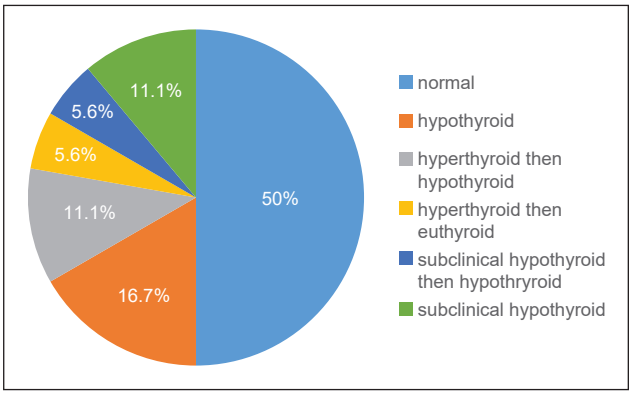

Figure 3. Types of thyroid dysfunction in ICI users. Of 18 patients, 50% had thyroid dysfunction which are 3 (16.7%) hypothyroid, 2 (11.1%) hyperthyroid then hypothyroid, 2 (11.1%) subclinical hypothyroid, 1 (5.6%) hyperthyroid then euthyroid and 1 (5.6%) subclinical hypothyroid then euthyroid. Finally, 6 (33.3%) patients had hypothyroid.

Table 3. Baseline characteristics in immune checkpoint inhibitors user

Demographic data	All ICI users
Age (mean ± SD), years	63.3 ± 12.5
Sex (%)	
Male	66.7%
Female	33.3%
Domicile region (%)	
Central	89.0%
West	5.5%
East	5.5%
Diagnosis (%)	
NSCLC	50.0%
RCC	22.2%
HCC	11.1%
Malignant melanoma	5.6%
Esophageal cancer	5.6%
Cecal cancer	5.6%
Comorbid disease (%)	27.8%
Hypertension	11.1%
Diabetes mellitus	11.1%
Cirrhosis	11.1%
Coronary artery disease	7.1%
Cerebrovascular disease	7.1%
Types of Immunotherapy use (%)	
Nivolumab	33.3%
Pembrolizumab	22.2%
Durvalumab	22.2%
Cemiplimab	16.7%
Atezolizumab	11.1%
Ipilimumab	5.6%
Combination immunotherapy	11.1%
anti-CTLA-4 + anti-PD1	5.6%
anti-PD1+ anti-PD1	5.6%
Duration of Immunotherapy before thyroid dysfunction (mean ± SD days)	61.3 ± 48.6

Baseline TFTs were unavailable in most of patients except for 2 patients with history of pazopanib use who showed sick euthyroid state with high anti-thyroid peroxidase (259 IU/ml) (n = 1) and subclinical hypothyroidism (n = 1) which resolved spontaneously before the start of immunotherapy.

Thyroid dysfunction occurred in 50% of patients. Three (16.7%) cases of hypothyroidism were found. Two (11.1%) patients initially had thyrotoxicosis followed by a state of hypothyroidism with 1 of the patients confirmed to have very low uptake in thyroid scintigraphy compatible with thyroiditis. One patient had transient hyperthyroidism then euthyroidism. Another 3 patients had subclinical hypothyroidism which turned to overt hypothyroidism (n = 1) and sick euthyroid syndrome (n = 1) on follow up (Figure 3). The mean duration of the first detected abnormal TFT was 61.3 ± 48.6 days (median 46 days, range: 14-162 days). Three of 4 patients with abnormal TFTs had positive thyroid antibodies. Conversion from hyperthyroid to hypothyroid state was in 92 and 42 days in patient numbers 3 and 10, respectively.

There was no relationship between sex, age, previous TKIs used, dose, type and duration of ICIs and thyroid dysfunction. No one had symptoms of thyroid dysfunction.

There was no interruption in ICI treatment in all patients, except 1 who received ipilimumab and cemiplimab who had hepatitis but no thyroid dysfunction.

The specific agents used were anti-PD1 for 12 patients (nivolumab, pembrolizumab and cemiplimab), anti-PD1L for 6 (atezolizumab and durvalumab), and anti-CTLA4 for 1 patient (ipilimumab). Thyroid dysfunction was found in 9 patients (6 on anti-PD1, 3 on anti-PDL1 and none from anti-CTLA4). Abnormal TFTs were observed on 50% of patients who received anti-PD1 and anti-PD1L. Hypothyroidism was observed with the combination of 2 anti-PD1 as in patient number 10 but not with the combination of ipilimumab with cemiplimab (anti-CTLA4 + anti-PD1) after 1 year follow up. Levothyroxine (LT4) replacement is needed in 55.5% of thyroid dysfunction. No other endocrinopathies such as type 1 diabetes or hypophysitis were observed in all ICI patients (Table 4).

There was no missing data in this study both in TKI and ICI group.

DISCUSSION

This is the first study to show the prevalence and associated factors of thyroid dysfunction from new anti-cancer therapies in a single tertiary center in Thailand. Assessments were done in out-patient department without other drugs affecting TFT to minimize conditions that may interfere with laboratory assays or yield sick euthyroid state. The prevalence of TKI-induced was 14.6% (n = 21) from 144 patients: subclinical hypothyroidism (11.1%), subclinical hyperthyroidism (14.5%) and overt hypothyroidism (9.5%). Most of these patients were asymptomatic or had mild clinical symptoms but did not warrant TKI interruption. Only 4 patients required LT4 treatment. Thyroid autoantibodies were positive in 4 out of 21 patients. The prevalence observed was lower than previously reported in the United States of about 40% with more overt hypothyroidism (26.8%) and subclinical hyperthyroidism (13.2%).[22] In other studies, prevalence ranged from 18-44%.[23] The differences may be due to the diagnosis and different type of TKIs used.

Table 4. Baseline characteristics and thyroid function test after immune checkpoint inhibitor use

No.	Age	Sex	Diagnosis	Previous TKI	Immunotherapy	FT4 (ng/dl)	FT3 (pg/ml)	TSH (mIU/ml)	Abnormal TFT	Onset abnormal TFT (days)	Anti-Tg (IU/ml)	Anti-TPO (IU/ml)	Treatment of Abnormal TFT	Cortisol (ug/dl)	FBS (mg/dl)	Survival
1	74	F	NSCLC	Erlotinib + Osimertinib	Atezolizumab	1.35	2.36	3.01	-	-	-	-	-	-	96	1
2	55	M	RCC	Pazopanib	Nivolumab	1.01	2.78	2.88	-	-	-	-	-	-	115	1
3	68	M	NSCLC	-	Durvalumab	2.18	5.18	0.009	Hyper-hypothyroid	46	327.50	94.82	Yes	8.20	-	1
4	83	F	HCC	Sorafenib	Nivolumab	0.73	2.99	16.21	Hypothyroid	14	-	-	-	-	-	1
5	70	M	RCC	Pazopanib	Nivolumab	0.31	1.62	>100	Hypothyroid	69	54.30	288	Yes	-	103	1
6	75	M	NSCLC	-	Atezolizumab	1.52	2.20	3.42	-	-	-	-	-	-	-	0
7	61	M	NSCLC	-	Durvalumab	1.21	2.99	0.104	Transient hyperthyroid	112	-	-	-	11.70	-	1
8	55	M	NSCLC	-	Durvalumab	1.60	3.42	2.33	-	-	-	-	-	-	-	1
9	30	F	NSCLC	-	Pembrolizumab	1.35	3.43	0.391	-	-	-	-	-	-	75	0
10	60	F	Malignant melanoma	-	Pembrolizumab + Nivolumab	1.83	4.21	<0.005	Hyper-hypothyroid	44	-	13.28	Yes	-	-	1
11	64	M	RCC	Sunitinib	Nivolumab	1.35	1.77	4.49	Sick euthyroid	-	-	-	-	-	-	0
12	61	M	Esophageal cancer	-	Nivolumab	0.99	2.27	14.30	Subclinical hypothyroid	14	<10	18.52	-	-	-	1
13	52	M	HCC	-	Durvalumab	1.39	2.74	12.18	Subclinical hypothyroid	28	-	-	-	-	97	0
14	78	F	Cecal cancer	-	Pembrolizumab	0.72	1.89	37.86	Hypothyroid	63	-	-	Yes	14.70	122*	0
15	78	F	RCC	Sunitinib	Pembrolizumab	1.32	1.61	1.66	Sick euthyroid	-	-	-	-	-	87	0
16	58	M	NSCLC	-	Ipilimumab + Cemiplimab	1.59	3.13	1.02	-	-	-	-	-	-	96	1
17	63	M	NSCLC	-	Cemiplimab	0.26	1.02	54.50	Hypothyroid	162	-	-	Yes	-	122	1
18	54	M	NSCLC	-	Cemiplimab	-	-	2.20	-	-	-	-	-	-	92	1

M = male, F = female, NSCLC = non-small cell lung cancer, RCC = renal cell carcinoma, HCC = hepatocellular carcinoma, TKI = tyrosine kinase inhibitors, FT4 = free thyroxine (normal 0.93-1.70 ng/dl), FT3 = free triiodothyronine (normal 2.00-4.40 pg/ml), TSH = thyroid stimulating hormone (normal 0.27-4.20 mIU/ml), anti-Tg = anti-thyroglobulin antibody (normal 0-115 IU/ml), anti-TPO = anti-thyroid peroxidase antibody (normal 0-34 IU/ml), TFT = thyroid function test, cortisol = morning cortisol, FBS = fasting blood sugar, survival 0 = dead, 1 = survive, * = previous type 2 diabetes

In this study, imatinib was used in 70.8% of patients mainly for CML and GIST while in other studies more usage of sunitinib, sorafenib and pazopanib for RCC or GIST were noted. The latter agents had more vascular endothelial growth factor (VEGF) inhibition resulting in more abnormal vasculature and thyroiditis from older reports. A majority of our patients were given imatinib due to government reimbursement policy and financial issues which limit the use of second or third line TKIs. The biphasic pattern of hyperthyroidism and hypothyroidism was not found in this study. However, subclinical hyperthyroidism observed in some of our patients may just be an initial manifestation of thyroid dysfunction which may subsequently convert to hypothyroidism. Hence, close follow up is needed.

The mean duration of TKI treatment in this study before TFT abnormality was 1215 ± 1332 days or 3.3 years which was longer than other previous studies of about 192-252 days. In other studies, hypothyroidism increases with more cycles of TKI used with the longest duration found at 92 weeks or 1.76 year.[24,25] We had low proportion of VEGF inhibitor use; hence, it might take more time to observe thyroid dysfunction. Ethnicity might be another factor. Most studies were done in Western countries and in Asia, the availability is limited to high VEFG inhibition drugs. Another reason was most of the dysfunctions had no or minimal clinical symptoms, hence no regular TFT monitoring was done but more cumulative exposures to TKI.

The number of TKIs used and dosing were not associated with thyroid dysfunction. Another difference was there were more males and fewer antibodies detected in patients with thyroid dysfunction suggesting a non-autoimmune process. Most of our patients live in the central region of the country and no relationship with thyroid dysfunction was seen. If iodine status were suspected to have a causal relationship, more research with patients in known iodine deficiency areas or confirmed low urinary iodine should be done.

The history of thyroid disease might not contribute to further abnormal TFTs from TKIs. One previous hypothyroid with below standard dose of imatinib use due to CKD required higher dose of LT4 (from 150 to 200 μg/day) but this may be due to poor compliance. Another patient with Hashimoto's thyroiditis and gefitinib use did not need additional LT4 dose and 1 case with thyroid nodule who received standard dose of imatinib did not have normal TFTs.

Contrast this with CKD and HBV infection which is associated with thyroid dysfunction. CKD could affect thyroid metabolism through decrease iodine excretion, decrease in thyroid binding protein, decrease peripheral conversion of thyroid hormones and increase in TSH.[24] There are no reports of chronic HBV being directly associated with thyroid dysfunction. However, in cirrhosis, there are free hormone and binding globulin changes. Some patients with interferon treatment also had more thyroid dysfunction.[26] In our study, there were no patients with decompensated cirrhosis or interferon treatment among those who have HBV infection. Thus, further study is required to confirm the association. Survival in our study was not significantly different between groups with and without thyroid dysfunction. Larger retrospective data however, showed more progression free survival, hypothesized from greater immune response, more cancer cells killed and more thyroid dysfunction.[26,27]

For ICIs, six patients had overt hypothyroidism, two of these patients had a transient thyrotoxicosis phase before hypothyroidism and another 1 had progression from subclinical to overt hypothyroidism. Transient hyperthyroidism suggests that thyroiditis might be the cause of thyroid dysfunction. One of our patients had confirmed thyroiditis by thyroid scan.

The incidence of thyroid dysfunction with ICIs in previous studies ranged from 5.9-21.0%[28,29] mainly from hypothyroidism which was lower than our study. The reported rate of overt hypothyroidism with anti-PD1 or anti-PD1L use was 6.5-7.5% which was lower than what we observed in our study with a prevalence of 50%.

The limitation of our study was the small number of patients and 66.6% of them were on anti-PD1 with more reported cases of hypothyroidism. Combination of ICIs had largest incidence of thyroid dysfunction of about 14.6% (REF). Thyroid autoantibodies, either anti-Tg and/or anti-TPO were positive in 23.0-40.0% of patients with abnormal TFTs.[30] In our study, 75% (4 out of 6 patients) had positive antibodies suggesting immune process involvement. History of TKI use before the start of ICI was not associated with thyroid dysfunction (2 patients, numbers 4 and 5). There was no previous thyroid disease identified among our ICIs population.

Baseline characteristics in patients with thyroid dysfunction were male sex (6 out of 9 patients), older (age 66.2 years, in normal TFT 60.3 years). The duration of ICI treatment to first thyroid dysfunction was 61.3 ± 48.6 days, earliest at 14 days and latest at 162 days, which were similar with prior published studies with mean duration 42 days.[31]

To our knowledge, this is the first study to collect thyroid dysfunction prevalence among cancer patients on TKIs and ICIs in single tertiary center of Thailand. There was no missing data. The limitation of our study was a cross-sectional design in TKIs and retrospective descriptive design in ICIs. There were no previous thyroid function tests at baseline before the start of TKI and ICI resulting in limitation of risk factors interpretation. The population included was below the calculated sample size, hence, the study might be underpowered. Lastly, the proportion of patients who received VEFG inhibition among TKIs was lower and anti-PD1 among ICIs were greater than in previous studies, thus, the prevalence and pattern of thyroid dysfunction might be different. Further research is therefore needed to clearly describe associations.

CONCLUSIONS

The prevalence of TKI-induced thyroid dysfunction was 14.6 %. The most common dysfunction was subclinical hypothyroidism and the majority of patients had no to mild symptoms. Treatment with levothyroxine was required in 4 patients. Prior thyroid dysfunction and thyroid autoimmunity did not show correlation. Some types of TKI were more likely to cause thyroid dysfunction but did not reach statistical significance. Male sex, CKD and HBV infection were significantly associated with thyroid dysfunction. We therefore recommend TSH monitoring for patients on TKIs with closer monitoring for patients on drugs with higher prevalence of thyroid dysfunction, e.g., sunitinib.

Thyroid dysfunction from ICIs was seen in 50% of patients which occurred at median duration of 46 days and 55.6% of them warranted levothyroxine replacement. Therefore, close monitoring in the first 6 months is recommended. No correlation between survival and thyroid dysfunction was seen. Further research is recommended to evaluate risk factors for higher thyroid dysfunction in Thailand.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
KC: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing-original draft preparation, Writing-review and editing, Visualization, Project administration, Funding acquisition; **KM:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing-original draft preparation, Writing-review and editing; **PT:** Conceptualization, Formal analysis, Investigation, Resources, Data Curation, Writing-original draft preparation, Writing-review and editing; **CD:** Conceptualization, Methodology, Validation, Formal analysis, Data Curation, Writing-original draft preparation; Writing-review and editing; Visualization, Supervision, Project administration, Funding acquisition.

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ORIGINAL ARTICLE

Malignancy in Thyroid Nodules with Bethesda III Category on Repeat Fine Needle Aspiration Biopsy*

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Abstract

Objectives. This study aimed to evaluate the risk of malignancy for nodules repeatedly classified as Bethesda category III on fine needle aspiration biopsy (FNAB).

Methodology. A chart review on a series of 59 patients seen with thyroid nodules who underwent both initial and repeat FNAB at the Diabetes, Thyroid and Endocrine Center of St. Luke's Medical Center, Quezon City was conducted. The Thyroid Registry was utilized to collect each patient's demographic and clinical characteristics, ultrasonographic features of thyroid nodules along with the cytopathologic and histopathologic results. The subclassification of atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) were retrieved from the cytopathology reports using the institution's electronic Healthcare-Results Management System.

Results. A total of 59 adult patients with thyroid nodules were included. Nodules which were initially AUS/FLUS turned out to be malignant on repeat FNAB in 38 patients with a prevalence of 64.41% (95% CI: 50.87-76.45%). There was no significant difference with regards to clinical, ultrasonographic and cytopathologic features of malignancy between benign and malignant nodules.

Conclusion. Findings of this study support surgical intervention as a reasonable option after a repeat Bethesda III classification on FNAB. However, the small sample size warrants confirmation in future studies with a representative sample of patients.

Key words: Bethesda III, AUS/FLUS, malignancy

INTRODUCTION

There are currently no guidelines available regarding management of repeatedly classified as Bethesda category III nodules or atypia of undetermined significance/ follicular lesion of undetermined significance (AUS/FLUS). A meta-analysis on the outcomes of repeat fine needle aspiration biopsy (FNAB) for AUS/FLUS thyroid nodules showed that the malignancy rate is about 40%.[1] To improve, diagnostic accuracy, some experts recommend core needle biopsy over repeat FNAB for this category.[2] There is no consensus regarding the treatment options for these patients, which can burden them with anxiety regarding additional costs and possible delays in definitive diagnosis and management.

The Bethesda System for Reporting Thyroid Cytopathology is a 6-category classification system created to standardize the interpretation of thyroid cytology. (Table 1) Categories I-VI include: non-diagnostic or unsatisfactory, benign, AUS/FLUS, follicular neoplasm/suspicious for follicular neoplasm (SFN), suspicious for malignancy (SFM), and malignant.[3] This system provides category-specific malignancy rates and recommends appropriate clinical management for each category.

Bethesda III has a predicted risk of malignancy that ranges from 10 – 30%.[3] The most frequently recommended management is repeat FNAB after 3 – 6 months. Even though the Bethesda System for Reporting Thyroid Cytopathology recommends repeat FNAB for AUS/FLUS,

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Table 1. Bethesda system for reporting thyroid cytopathology: diagnostic categories and risk of malignancy[3]

Bethesda category	Cytopathologic category	Approximate expected frequency	Malignancy rate	Suggested treatment (Prior to availability of molecular testing)
I	Nondiagnostic/inadequate	5-11	1-4	Repeat FNA
II	Benign	55-74	0-3	US follow up
III	Atypia/follicular lesion of undetermined significance	5-15	5-15	Repeat FNA in 3-6 months
IV	Follicular neoplasm/suspicious for FN	2-25	15-30	Lobectomy
V	Suspicious for malignancy	1-6	60-75	Lobectomy or thyroidectomy
VI	Malignant	2-5	97-99	Total thyroidectomy

the American Thyroid Association (ATA) is less clear with its weak recommendation,[4] while the American Association of Clinical Endocrinology (AACE)/Associazione Medici Endocrinologi (AME)/European Thyroid Association (ETA) favor surgery and recommend that repeat FNAB should not be performed because of the possibility of confusing results.[5] Recent studies have shown a higher risk of malignancy and higher rates of immediate surgery.[6] Although many studies have suggested a different method for management of Bethesda III nodules, such as incorporation of data from clinical history, laboratory results and ultrasonographic findings,[7-10] repeat FNAB is still frequently performed. The variability in the reported malignancy rates of repeat AUS/FLUS suggests diagnostic heterogeneity.[6,11-16] Further studies may be needed to determine the actual risk of malignancy, since a histopathologic diagnosis is only available for patients who underwent surgery, and to determine the appropriate management of an AUS/FLUS cytopathologic diagnosis.

Molecular testing has been suggested to provide additional information for patients with Bethesda III nodules. Among the several Raf kinase isoforms, the B-type Raf kinase (BRAF) is the strongest activator of the downstream regulated kinase signaling pathway and is associated with early tumorigenesis and aggressive behavior of papillary thyroid carcinoma.[17] In a 2015 survey of clinical practice patterns in the United States, 38.8% of 820 respondents would obtain molecular testing after an initial AUS/FLUS result.[18] Its high negative predictive value of at least 95% may be used by physicians as basis for deferring surgery.[19] Molecular testing was considerably more cost-effective than diagnostic lobectomy for Bethesda III nodules.[20] It has been adopted in developed countries as it has reduced the number of surgeries with indeterminate cytopathology. Nonetheless, its routine application in our healthcare setting cannot be advocated due to its cost per case base and availability.

To date, there are a few studies that have investigated on nodules repeatedly classified as Bethesda III and there is still no consensus for the management of these cases. Therefore, this study aimed to evaluate the risk of malignancy for repeat Bethesda III nodules on FNAB and explore its correlation with the demographic and clinical patient characteristics, which may help assist in developing guidelines on the management of such nodules.

OBJECTIVES

General objective

To determine the proportion of malignancy of thyroid nodules repeatedly classified as Bethesda category III on FNAB

Specific objectives

1. To describe the clinical and ultrasonographic features of repeat Bethesda category III nodules on FNAB
2. To compare the demographic, clinical, ultrasonographic and cytopathologic characteristics between malignant cases versus benign.

METHODOLOGY

Study design and population

The design was a chart review on a series of 59 patients seen with thyroid nodules who underwent both initial and repeat FNAB at the Diabetes, Thyroid and Endocrine Center (DTEC) of St. Luke's Medical Center Quezon City (SLMC-QC), Philippines. Patients included were those who received a cytopathologic diagnosis of Bethesda Category III or AUS/FLUS at least twice within a span of 1 year from June 2017 to December 2021. Relevant clinical data of the included participants were taken from the DTEC Thyroid Registry. No patient contact was done and data were gathered retrospectively. The researchers also checked the medical records from the private Endocrinology clinics for patients who had 2 FNAB results of AUS/FLUS but had no record of surgery done at the same institution, to check for any histopathological result. Patient records which could not be retrieved were considered lost to follow-up.

Operational definition

- Fine Needle Aspiration Biopsy (FNAB) – gold standard diagnostic tool for thyroid nodules, indicated for the following:[4]
  - Patients with clinical signs of thyroid cancer
  - Nodules >1 cm with at least 2 ultrasound criteria for malignancy
  - Nodules of any size with extracapsular extension or indeterminate cervical lymph nodes
  - Nodules of any size in patients with a history of neck radiation

Table 2. Sonographic patterns, estimated risk of malignancy, and fine needle aspiration guidance for thyroid nodules[3]

Sonographic pattern	Ultrasound features	Estimated risk of malignancy	FNA size cutoff (largest dimension)
High suspicion	Solid hypoechoic nodule or solid hypoechoic component of a partially cystic nodule with one or more of the following features: irregular margins (infiltrative, micro lobulated), microcalcifications, taller than wide shape, rim calcifications with small extrusive soft tissue component, evidence of ETE.	>70–90	Recommend FNA at ≥1 cm
Intermediate suspicion	Hypoechoic solid nodule with smooth margins without microcalcifications, ETE, or taller than wide shape.	10–20	Recommend FNA at ≥1 cm
Low suspicion	Isoechoic or hyperechoic solid nodule, or partially cystic nodule with eccentric solid areas, without microcalcification, irregular margin or ETE, or taller than wide shape.	5–10	Recommend FNA at ≥1.5 cm
Very low suspicion	Spongiform or partially cystic nodules without any of the sonographic features described in low, intermediate, or high suspicion patterns.	<3	Consider FNA at ≥2 cm (Observation)
Benign	Purely cystic nodules (no solid component)	<1	No biopsy

  - History of well-differentiated thyroid carcinoma in more than 2 first-degree relatives
  - Medullary thyroid carcinoma or multiple endocrine neoplasia (MEN) type 2
  - Increased calcitonin levels
- Thyroid Nodule – an abnormal growth of thyroid cells that forms a lump within the thyroid gland. According to the 2015 ATA Guidelines, it can be further described based on ultrasound findings and FNA results shown in Table 2
- Bethesda III (Atypia/Follicular Lesion of Undetermined Significance) Subclassification:[21]
  - Cytologic (Nuclear Atypia) – described as having mild nuclear enlargement, membrane irregularities such as wrinkles or grooves, overlapping, or chromatin clearing
  - Architectural Atypia – described as having low cellularity, absent or minimal colloid and microfollicles (including oncocytic changes)

Study procedures and data gathering

The Thyroid Registry of DTEC of SLMC-QC was utilized to collect each patient's demographic and clinical characteristics, ultrasonographic features of thyroid nodules (sonographic pattern, size, echogenicity, presence of vascularity and calcifications), along with the cytopathologic and histopathologic results. The ultrasound results were read by different radiologists from different institutions which were subsequently recorded in the DTEC Thyroid Registry in a standardized tabular format. The subclassification of the AUS/FLUS were retrieved from the comments section of the cytopathology reports using the electronic Healthcare-Results Management System of SLMC-QC. The slides were read by different cytopathologists from SLMC_QC, hence the cytologic diagnosis had a standardized reporting format, as it came from 1 institution.

Sampling methodology

The researcher utilized total enumeration technique wherein all eligible patients were included in the study. Based on the Thyroid Registry, only 59 patients satisfied the inclusion and exclusion criteria.

Statistical analysis

Stata MP version 17 (Stata Corp LLC, College Station, TX, US) was used for data processing and analysis. No imputation of missing data was performed. Mean and standard deviation or median and interquartile range were used to describe continuous variables depending on the data distribution. Shapiro Wilk's test was used to assess normality of data. Frequency and percent distribution were used to describe categorical variables. Comparison by histopathologic result was performed using Chi-square test or Fisher's exact test for categorical variables, and independent t-test or Mann Whitney U test for continuous variables.

An exploratory analysis on the relationship of malignancy with the patient characteristics using simple logistic regression analysis was implemented. Estimates of crude odds-ratio and 95% confidence interval were reported. P values ≤0.05 were considered statistically significant.

Ethical considerations

The Clinical Protocol and all relevant documents were reviewed and approved by the SLMC-QC Institutional Ethics Review Committee. Patient confidentiality was respected by ensuring the anonymity of patient records. Each patient document was CODED and did not contain any identifying information in order to ensure confidentiality. All study data were recorded and investigators were responsible for the integrity of the data i.e., accuracy, completeness, legibility, originality, timeliness and consistency.

RESULTS

A total of 101 patients had nodules that were classified as Bethesda III or AUS/FLUS on both initial and repeat FNAB from the Thyroid Registry from June 2017 to December 2021. Of the 101 patients, 59 underwent surgery, 6 were advised but refused surgery, 9 were advised repeat FNAB, and 27 were lost to follow-up (Figure 1).

The 59 patients who underwent surgery after repeat Bethesda III on FNAB were included in this study. The median time from repeat FNAB to surgery was 1.88 months

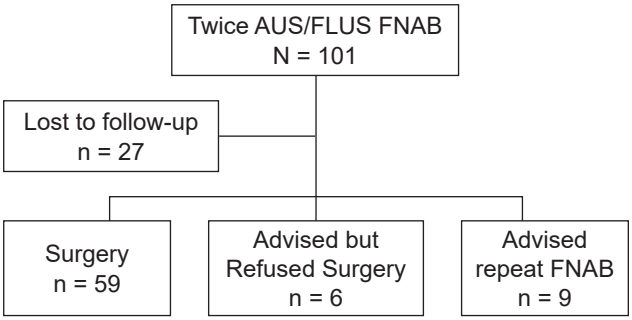

Figure 1. Management of repeat Bethesda III nodules at St. Luke's Medical Center from June 2017 to December 2021.

(range: 0.26-30.60 months). Thirty-eight patients had thyroid cancer based on histopathology, having a prevalence of 64.41% (95% CI: 50.87-76.45%).

The baseline demographic and clinical characteristics of patients by histopathology result are presented in Table 3. The mean age was 46.64 years old (range 21 to 70 years old) and the majority of the patients were females (86%). A family history of thyroid dysfunction and malignancy were reported by 14% and 8% of patients, respectively. The median thyroid stimulating hormone (TSH) was 1.25 mIU/L (range 0.27-22.66) and a majority (87%) had normal TSH. There were no reported obstructive symptoms such as hoarseness, dyspnea and dysphagia from the patients. Ten percent reported taking low dose levothyroxine for the thyroid nodules. The patients were homogenous in demographic and clinical characteristics.

There was no significant difference between the 2 groups (benign vs papillary thyroid carcinoma cytopathology) with regards to sonographic pattern, thyroid nodule size, vascularity, calcification and echogenicity (Table 4). More than half (53%) of the patients had intermediate to high sonographic pattern combined. Consequently, more than half (53%) of the nodules were hypoechoic, 44% were isoechoic and 3% were hyperechoic. The majority (90%) had a thyroid nodule size of ≥1 cm. Ten percent of nodules were vascular and 25% percent were positive for calcifications.

There was no significant difference between the 2 groups (benign vs papillary thyroid carcinoma cytopathology) with regards to the subclassification of AUS/FLUS on FNAB (Table 5). Most patients had cytologic atypia on the first (71%) and second (63%) FNAB.

Table 3. Demographic and clinical characteristics of Bethesda III patients by histopathologic result (n = 59)

Characteristics	All patients (n = 59), N(%)	Malignant (n = 38), N(%)	Benign (n = 21), N(%)	*P*
Age (in years), mean ± SD	46.64 ± 11.52	46.68 ± 12.34	46.57 ± 10.14	0.972
Sex				0.438
Female	51 (86)	34 (67)	17 (33)	
Male	8 (14)	4 (50)	4 (40)	
Past medical history				
Malignancy				
Without	54 (92)	35 (65)	19 (35)	1.000
With	5 (8)	3 (60)	2 (40)	
Malignancy: Head and neck				
Without	59 (100)	38 (100)	21 (100)	
With	0	0	0	
Thyroid disease				
Without	58 (98)	37 (64)	21 (36)	1.000
With	1 (2)	1 (100)	0	0.438
Family history: Thyroid dysfunction				
No	51 (86)	34 (67)	17 (33)	0.438
Yes	8 (14)	4 (50)	4 (50)	0.646
Family history: Thyroid malignancy				0.646
No	54 (92)	34 (63)	20 (37)	
Yes	5 (8)	4 (80)	1 (20)	
TSH (in mIU/L)[a]				
median	1.25	1.24	1.63	0.7840
IQR	0.81-1.94	0.86-1.9	0.69-2.69	
Low	5 (10)	3 (60)	2 (40)	0.085
Normal	44 (87)	32 (73)	12 (27)	-
High	2 (4)	0	2 (100)	
History of radiation				
No	59 (100)	38 (64)	21 (36)	1.000
Yes	0	0	0	
Presence of obstructive symptoms				1.000
No	54 (92)	35 (65)	19 (35)	0.733
Yes	5 (8)	3 (60)	2 (40)	
Thyroid Medications				
No	49 (83)	32 (65)	17 (35)	
Yes	10 (17)	6 (60)	4 (40)	

[a]Only 51 patients have data for TSH

Table 4. Ultrasonographic features of Bethesda III patients by histopathologic result (n = 59)

Characteristics	All patients (n = 59), N(%)	Malignant (n = 38), N(%)	Benign (n = 21), N(%)	*P*
Sonographic pattern				0.584[a]
Very low/low	28 (48)	17 (61)	11 (39)	
Intermediate	15 (25)	9 (60)	6 (40)	
High	16 (27)	12 (75)	4 (25)	
Size of thyroid nodule				0.407[b]
≥1 cm	53 (90)	33 (62)	20 (38)	
<1 cm	6 (10)	5 (83)	1 (17)	
Vascularity				1.000[b]
Non-vascular	53 (90)	34 (64)	19 (36)	
Vascular	6 (10)	4 (67)	2 (33)	
Calcification				0.403[a]
Negative	44 (75)	27 (61)	17 (39)	
Positive	15 (25)	11 (73)	4 (27)	
Echogenicity				0.901[b]
Isoechoic	26 (44)	17 (63)	10 (37)	
Hypoechoic	31 (53)	20 (67)	10 (33)	
Hyperechoic	2 (3)	1 (50)	1 (50)	

Table 5. Cytopathologic features of Bethesda III patients by histopathologic result (n = 59)

Characteristics	All patients (n = 59), N(%)	Malignant (n = 38), N(%)	Benign (n = 21), N(%)	*P*
First FNAB				0.108[a]
Cytologic atypia	42 (71)	29 (69)	13 (31)	
Architectural atypia	5 (8)	1 (20)	4 (80)	
Both cytologic and architectural atypia	12 (20)	8 (67)	4 (33)	
Second FNAB				0.211[a]
Cytologic atypia	37 (63)	24 (65)	13 (35)	
Architectural atypia	6 (10)	2 (33)	4 (67)	
Both cytologic and architectural atypia	16 (27)	12 (75)	4 (25)	

The results on insufficient evidence of differences between the 2 groups were consistent with the lack of statistical significance in the crude association of the patient characteristics with malignancy (Table 6).

DISCUSSION

AUS/FLUS is a heterogenous cytopathologic category that was neither definitively benign nor definitively malignant.[22] Three possible management options are recommended by current guidelines for AUS/FLUS follow-up: (1) Clinical observation; (2) Repeat FNAB with management being based on the result of the last exam; or (3) Surgery (lobectomy/thyroidectomy).[11] The approach to the management of these Bethesda III nodules differs among physician-specialists, although the majority adhere to the clinical practice guidelines of the ATA.[23] There were a few studies that have investigated on repeatedly classified as Bethesda III nodules and there is no consensus on the treatment options for these patients.

In this study, a repeat Bethesda III on FNAB had a 64% risk of malignancy. This is lower than the risk of malignancy (73.1%) after repeat Bethesda III by Yoo et al.[24] On the other hand, the malignancy risk in this study is higher than the risks of malignancy after repeat Bethesda III in the studies of Bayona et al.,[1] Wong et al.,[16] Ogmen et al.,[12] and Ho et al.,[6] at 40%, 39%, 32.4% and 26.3%, respectively. Whether this higher risk reflects a more aggressive nature of lesions among Filipinos remains debatable. It is important to note that these reported rates are higher than the risk of malignancy (5-15%) of Bethesda III as adopted by the Bethesda System.

The risk of malignancy in this study may also be higher since only those patients who underwent surgery were included. Surgery was most probably performed on patients considered high risk for malignancy. Those patients who were advised but refused surgery, advised repeat FNAB and lost to follow-up were excluded. Thereby, a 64% risk of malignancy in this study may reflect the higher baseline risk of patients and may not estimate the true rate of malignancy after repeat Bethesda III among patients with lower baseline risk.

With the wide range of malignancy rates found in these studies, the variability in the reported risks may be due to different rates of repeat FNAB versus surgery from the conflicting management guidelines for AUS/FLUS. In the Philippines, the survey of Abelardo et al., revealed that the management of patients with AUS/FLUS diagnosis is heterogenous within and across different specialties, at the same individualized depending onthe patient's clinical and ultrasonographic features.[23] Greater management differences in practice may be seen after repeat Bethesda III owing to the lack of concrete guidelines. In addition, the variability might come from the heterogeneity and subjectivity of the reported AUS/FLUS being read, since technical issues including adequacy of sample and optimization of cellular preservation from slide preparation may have greatly affected the results.[25,26] This highlights the value of interpreting AUS/FLUS results with caution

and combining it with clinical correlation to arrive at a clinical decision.

There were no clinical characteristics correlated with malignancy in this study, which is in congruence with the previous studies. In several studies, age and sex were not considered predictors of malignancy for thyroid nodules with AUS/FLUS.[8-10] However, other studies found that the malignancy rate was higher in younger patients.[12,27] Furthermore, neither a family history of papillary thyroid carcinoma nor a history of radiation exposure increases the risk of malignancy in patients with AUS/FLUS.[8-10] Even though several known clinical risk factors in patients with thyroid nodules for thyroid cancer include immobility with swallowing, pain, cough, voice change, growth and lymphadenopathy, these have not been included in multivariate analyses of ultrasonographic features and thyroid cancer risk.[3]

Table 6. Exploratory results on the association of patient characteristics with malignancy

Characteristics	Crude OR (95% CI)	*P*
Age (in years)	1.00 (0.96-1.05)	0.967
Sex		
Female	Ref	Ref
Male	0.51 (0.12-2.11)	0.351
Past medical history		
Thyroid disease		
Without	Ref	Ref
With	1.72 (0.07-44.10)	0.743
Family history: Thyroid dysfunction		
No	Ref	Ref
Yes	0.51 (0.12-2.11)	0.351
Family history: Thyroid malignancy		
No	Ref	Ref
Yes	1.78 (0.26-12.24)	0.557
Smoking status		
No	Ref	Ref
Yes	0.53 (0.09-3.35)	0.504
Alcohol consumption		
No	Ref	Ref
Yes	1.26 (0.31-5.07)	0.746
Presence of obstructive symptoms		
No	Ref	Ref
Yes	0.77 (0.14-4.27)	0.764
Thyroid medications		
No	Ref	Ref
Yes	0.78 (0.20-2.96)	0.712
Sonographic pattern		
Very low/low	Ref	Ref
Intermediate	0.96 (0.28-3.33)	0.949
High	1.83 (0.49-6.76)	0.368
Size of thyroid nodule		
≥1 cm	Ref	Ref
<1 cm	2.24 (0.34-14.81)	0.401
Vascularity		
Non-vascular	Ref	Ref
Vascular	1.02 (0.20-5.26)	0.984
Calcification		
Negative	Ref	Ref
Positive	1.63 (0.47-5.64)	0.443
Echogenicity		
Isoechoic	Ref	Ref
Hypoechoic	1.17 (0.40-3.41)	0.771
Hyperechoic	0.60 (0.06-6.54)	0.675
First FNAB		
Cytologic atypia	Ref	Ref
Architectural atypia	0.11 (0.01-1.10)	0.061
Both cytologic and architectural atypia	0.90 (0.23-3.52)	0.876
Second FNAB		
Cytologic atypia	Ref	Ref
Architectural atypia	0.27 (0.04-1.68)	0.161
Both cytologic and architectural atypia	1.63 (0.44-6.07)	0.470

It has been well-established that a thyroid nodule has a highly suspicious sonographic pattern when it has these features: solid consistency, hypoechogenicity with one or more of the following; irregular margins (infiltrative, microlobulated, or spiculated), microcalcifications, taller than wide shape, disrupted rim calcifications with small extrusive soft tissue component, and evidence of extrathyroidal extension.[3] Nodules measuring ≥1 cm with this sonographic pattern should undergo diagnostic FNAB to confirm malignancy.[3] Nodule size has not been correlated with malignancy in most studies.[7,8,10] This study showed that these high-risk ultrasound features were not statistically different between benign and malignant nodules. However, further studies of a larger scale are necessary to confirm these non-significant findings.

In cytopathology reports, AUS/FLUS can be classified into 3 categories: cytologic (nuclear) atypia, architectural atypia, or both. Some of the common situations where AUS/FLUS is used are in samples with occasional follicular cells that have enlarged, pale, grooved, nuclei in an otherwise benign appearing aspirate (cytologic atypia); prominent microfollicles in a sparsely or only moderately cellular aspirate (architectural atypia); or when evaluation of follicular cell atypia is less than optimal because of an artifact produced from sample preparation.[28]

Many studies reported that the malignancy rate of cytologic atypia is higher than that seen with architectural atypia. In the study of Gan et al., cytologic atypia had a malignancy rate of 36.8% compared to 14.7% in architectural atypia.[29] Furthermore, the meta-analysis by Ahn et al., revealed the overall malignancy rate of cytologic atypia (24.3 – 65.8%) to be significantly higher than in architectural atypia (5.95 – 38.8%).[30] In spite of that, this study showed that the subclassification of AUS/FLUS on FNAB was not statistically different between benign and malignant nodules. As mentioned earlier, the interpretation of FNAB results may be influenced by the subjective reading of the individual cytopathologists, which could have produced the heterogeneity.[24,25] Similarly, the non-significant findings require confirmation in future studies with larger sample size.

Limitations

This study was limited to a chart review on a series of patients. There may have been a selection bias since patients with thyroid nodules initially classified as Bethesda III who were eventually lost to follow-up were excluded. In addition, the individualized approach of the physician

and preference of the patient have affected the decision to proceed with surgery or not after a repeat Bethesda III. In the same way, both the initial and repeat cytopathologic reports were read by different cytopathologists, and is subjective.

Due to the small population of 59 patients, the findings of this study cannot be used to make inferences to a larger population, and thus warrants confirmation in future studies with a representative sample of patients.

CONCLUSION AND RECOMMENDATION

Based on the results of this study, a second FNAB result of AUS/FLUS carries a 64% risk of malignancy. This suggests an elevated risk of malignancy compared with a single FNAB result of Bethesda III (5 – 15%). Therefore, this study supports surgical intervention (lobectomy/ thyroidectomy) as a reasonable option after a second Bethesda III classification on FNAB as recommended by AACE/AME/ETA.

The researchers recommend future studies with larger sample size to confirm the findings of this study. It is also recommended to explore the significance of cytologic and architectural atypia to the risk of malignancy. Therefore, we likewise recommend to include a pathologist be a study co-author.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
JLN: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration; **LME:** Conceptualization, Methodology, Validation, Writing – original draft preparation, Visualization, Supervision, Project administration; **OAD:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration.

Author Disclosure
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ORIGINAL ARTICLE

Cardiac and Metabolic Effects of Bariatric Surgery Among Obese Patients in a Malaysian Tertiary Hospital: A 6-month Prospective Cohort Study

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Abstract

Objective. Obesity is known to be associated with left ventricular diastolic dysfunction due to its effect on blood pressure and glucose tolerance. We aimed to investigate whether weight loss after bariatric surgery might improve diastolic dysfunction through in-depth echocardiographic examination.

Methodology. We recruited twenty-eight patients who were about to undergo bariatric surgery by purposive sampling. They underwent echocardiography at baseline and 6 months after surgery with a focus on diastolic function measurements and global longitudinal strain (GLS). They also had fasting serum lipid and glucose measurements pre- and post-surgery.

Results. The mean weight loss after surgery was 24.1 kg. Out of the 28 subjects, fifteen (54%) initially had diastolic dysfunction before surgery. Only two had persistent diastolic dysfunction 6 months after surgery. The mean indexed left atrial volume 6 months post-surgery was 27.1 from 32 ml/m^2 prior to surgery. The average E/e' is 11.78 post-surgery from 13.43 pre-surgery. The left ventricular GLS became (-)25.7% after surgery from (-)21.2% prior to surgery. Their post-surgery fasting serum lipid and glucose levels also showed significant improvement.

Conclusion. Our study reinforced the existing evidence that bariatric surgery significantly improved echocardiographic parameters of diastolic function and left ventricular global longitudinal strain, along with various metabolic profiles.

Key words: bariatric surgery, obesity, diastolic function, GLS

INTRODUCTION

Obesity is defined as body mass index (BMI) more than 30 kg/m^2 by World Health Organization and BMI >27.5 kg/m^2 by the Malaysian clinical practice guidelines (CPG) 2004. Malaysia was declared as the most obese country in Asia in 2014. Half of the population is obese. Obesity has been identified as a significant health issue worldwide. It is associated with detrimental effects on the cardiovascular system leading to morbidity and mortality.[1] In general, obesity is an independent risk predictor for heart failure.[2] It is closely related to cardiovascular risk factors such as hypertension, type 2 diabetes mellitus, and dyslipidemia, which directly affects cardiac structure and function. Excess body fat increases preload and afterload due to hyperdynamic circulation, chronic volume overload, and an increase in peripheral resistance.[3,4] The long-term care of obesity can be debilitating for both the patient and healthcare practitioner. The first bariatric surgery in 1954 by Dr A.J. Kremens was a jejunoileal bypass, which was later altered in 1964 by adding a jejuno-colic shunt. Several types of bariatric surgery methods were introduced throughout the years, with the latest being Roux-en-Y bypass and sleeve gastrectomy. Bariatric surgery is still the most effective weight loss intervention with more persistent benefits.[5]

Dyslipidemia plays a major role in the progression of cardiovascular disease in obesity. It is defined as an elevation in fasting blood total cholesterol, which may or may not be associated with elevated blood triglycerides (TG).[6] Dyslipidemia is further classified by the National Cholesterol Education Program (NCEP) into subtypes of lipoproteins, namely Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), and Triglycerides (TG).[7] In a retrospective study among patients who underwent Roux-en-Y gastric bypass, serum total cholesterol, TG, and LDL levels improved in all patients within 6 months after surgery leading to the discontinuation of their lipid-

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lowering agents. HDL level had a slower improvement, reaching the desired target within 12 months after surgery.[8]

Left ventricular (LV) diastolic dysfunction is also commonly associated with obesity. A condition that reflects an impairment in the filling properties of the LV, diastolic dysfunction has been demonstrated to predict future progression to heart failure.[9-11] Diastolic dysfunction refers to the inability of the heart chamber to properly fill with blood during the diastolic phase of the cardiac cycle. This is caused by inadequate relaxation of the ventricles during diastole by both active and passive processes occurring at the level of the myocyte, extracellular matrix, and left ventricular chamber. With regards to the obese population, it was postulated that the negative effects of visceral fat on diastolic function can be accounted for by concentric LV remodeling, elevated myocardial triglyceride levels, and impaired metabolism.[12]

There have been few studies on the effect of bariatric surgery on diastolic dysfunction. A study by Kurnicka et al., looked at the improvement of left ventricular diastolic function and morphology in young women with morbid obesity six months after bariatric surgery. Echocardiography was performed pre and post-surgery. Among the parameters assessed were mitral peak early (E) and atrial (A) velocities, E-deceleration time (DcT), pulmonary vein S, D and A reversal velocities, peak early diastolic mitral annular velocities (E') and E/E'. The study showed a significant reduction in LV wall thickness and LV mass (mean 183.7 to 171.5 g, $p = 0.001$).[13]

In the Asian population, diastolic dysfunction is detected at a lower BMI level, as compared to Caucasians.[14-21] This was seen in the study by Onzo et al., in 2020 which looked at the impact of ethnicity on cardiac adaptation. The study revealed that body size, left ventricular mass, wall thickness, trabeculation, genotype, and phenotype played a role in deciding one's cardiac morphology.[22]

Global longitudinal strain (GLS) is an emerging echocardiographic parameter that may predict cardiovascular outcomes and subclinical heart failure. Among the three main strains (longitudinal, radial and rotational), longitudinal strain is more commonly assessed as it is the first to be affected compared to the other strains. However, recent reports showed that the reduction of GLS is independent of heart failure and ejection fraction.[23] Multiple studies have shown improvements in GLS post-bariatric surgery. In a study done by Frea et al., in 2020 involving 40 patients who underwent bariatric surgery, there was an improvement in GLS 10 months after surgery.[24]

In 2019, a meta-analysis of 7 randomized controlled trials examining the outcome of bariatric surgery versus medical treatment for type 2 diabetes mellitus showed that bariatric surgery is superior and has a more persistent effect on type 2 diabetes mellitus remission compared to medical management.[25]

There has not been a comprehensive study looking into the diastolic parameters among patients who underwent bariatric surgery. Hence, we aimed to assess the echocardiographic parameters of diastolic dysfunction, together with blood lipid and glucose levels pre- and post-bariatric surgery, thus giving us an overview of the long-term outcome of bariatric surgery in Malaysia.

METHODOLOGY

Study design and participants

We performed a prospective observational study from January 2022 to August 2022, recruiting patients from the surgical and cardiology clinics at our center before they underwent bariatric surgery. Subjects were recruited by purposive sampling. We included patients with ages between 18 and 50 years old, BMI more than 27.5 kg/m^2, and who were about to undergo either a Roux-en-Y or sleeve gastrectomy. Patients were excluded if they had pre-existing ischemic heart disease, chronic kidney disease (CKD), end-stage renal failure (ESRF), congestive heart failure, atrial fibrillation, valvular heart disease and poor echo window for analysis. They were also excluded if there were regional wall motion abnormalities on their baseline echocardiogram, congenital heart disease, left bundle branch block (LBBB), LVEF of less than 35%, and obvious LVH with LV wall thickness of more than 1.3 cm in the initial echo. For this study, Valsalva maneuver was not done.

Laboratory measurements

The laboratory tests were done at the Department of Pathology of our institution. Fasting serum lipids, fasting blood sugar (FBS), and HbA1c were taken before and 6 months after surgery.

Sample size

We used a one-proportion cross-sectional sample size method, in which the proportion of the studied population is about 1%, based on the expected prevalence of cardiac dysfunction among obese patients 6 months after bariatric surgery, in which a meta-analysis by Veldhuisen et al., showed an incidence between 0.4 to 9.9%[26].

$$N = \frac{Z^2_{\alpha/2} * p * (1 - p) * DEFF}{d^2}{}_b$$

α = probability of type I error = 0.05
p = prevalence proportion = 0.01
DEFF = estimated Effect Size (Usually 1-4) = 1
d = desired level of absolute precision = 0.05
N = Required sample size

The calculated minimum number of sample size is sixteen. Assuming an attrition rate of at least 40%, we recruited a total of 28 patients.

Echocardiographic data

The echocardiographic study was done by a single qualified and certified operator using an ultrasound machine Epic 7C (Philips Ultrasound, Netherlands) system equipped with a 1.6-3.2 MHz phased-array transducer. All the subjects underwent echocardiography prior to and six months after bariatric surgery. The echo parameters used to determine diastolic dysfunction are Left Atrial Volume Index (LAVI) <25 ml/m^2, Average E/e'>14, Septal e' velocity <7 cm/s or lateral e' velocity <10 cm/s, TR velocity >2.8 m/s. E wave, E/A and LV strain percentage assessments are also part of the diastolic dysfunction assessment. If less than 50% of the above criteria are met, the diastolic function is considered normal. Diastolic dysfunction is deemed indeterminate if 50% of the above criteria are met. Diastolic dysfunction is regarded as abnormal if more than 50% of the above criteria are met. Grading for diastolic dysfunction was also determined pre-surgery. The algorithm to determine the grading of diastolic dysfunction is based from the American Society of Echocardiography April 2019.

Ethics statement

This study protocol was reviewed and approved by our institutional review board for ethics. Written consent was obtained from all patients involved in the study prior to their participation. All patients were counselled on the risks and benefits of their involvement in the study.

Statistical analysis

Categorical variables were described by frequency and percentage. Continuous variables were described using mean and standard deviation for normally distributed variables. The median and interquartile range (IQR) were reported if the distribution was not normal. Descriptive statistics, such as minimum and maximum values, were reported when necessary. Normality of the data was examined using a histogram (approximately bell-shaped), skewness (within -1 to 1), and kurtosis (within -3 to 3).

For normally distributed datasets, paired t-test was used to compare the differences in the measurements before and after the surgery. Wilcoxon signed-rank test was applied if the distribution of the differences was skewed. A p-value less than 0.05 is considered statistically significant.

McNemar test was used to compare the proportion of diastolic dysfunction before and after bariatric surgery. A p-value less than 0.05 was considered statistically significant. All analyses were performed using SPSS (IBM Corp. Released 2013 IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

Table 1. Distribution of subjects according to demographic and clinical characteristics, n = 28

Variables	Overall
Participants (N,%)	28
Gender (N,%)	
Male	8 (29)
Female	20 (71)
Ethnicity (N,%)	
Malay	26 (92)
Chinese	1 (4)
Indian	1 (4)
Hypertension (N,%)	
Yes	13 (46)
No	15 (54)
Diabetes (N,%)	
Yes	9 (32)
No	19 (68)
Dyslipidemia (N,%)	
Yes	15 (54)
No	13 (46)
Types of Surgery	
Roux-en-Y	9 (32)
Sleeve gastrectomy	19 (68)

* Categorical data present as frequency (percentage).

RESULTS

Out of fifty-two patients initially screened, 28 patients met the inclusion and exclusion criteria. All patients completed the study follow-up.

Eight (29%) subjects were males and 20 (71%) were females. Thirteen (46%) have hypertension, 9 (32%) have diabetes mellitus, and 15 (54%) have dyslipidemia (Table 1).

All patients had significant weight loss after surgery as shown in Table 2 and Figure 1. The mean BMI was 44.2 kg/m^2 pre-surgery and 35.6 kg/m^2 post-surgery, with a mean difference of 8.6 kg/m^2 (7.08 – 10.09). The mean systolic blood pressure (SBP) was 141 mm Hg pre-surgery and 121 mmHg post-surgery. There was a significant reduction in SBP with a mean difference of 19.8 mmHg (11.4 – 28.2).

For fasting serum lipid and glycemic control, the results showed statistically significant reductions, as seen in Table 2 and Figures 2 and 3.

For the echocardiographic measurements, there was a statistically significant improvement in all the parameters among the subjects with diastolic dysfunction at baseline as shown in Table 3 and Figure 4.

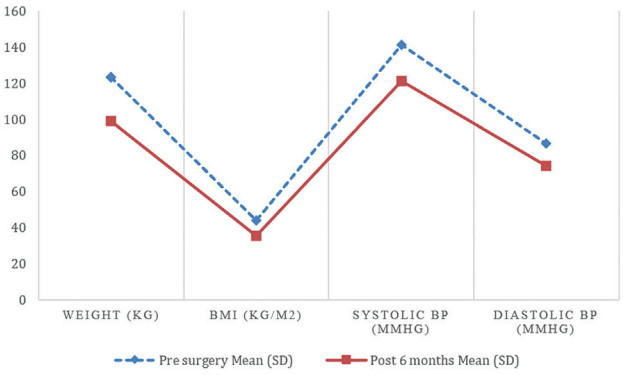

Figure 1. Graph showing comparison between anthropometric and blood pressure parameters before and after bariatric surgery.

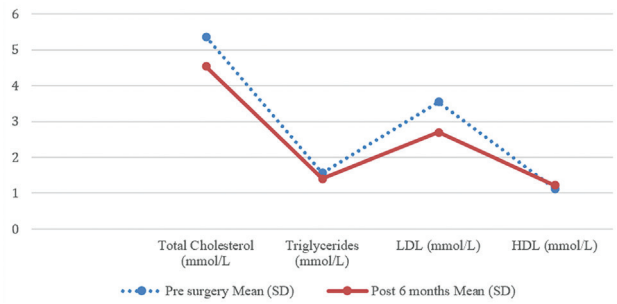

Figure 2. Graph showing comparison of lipid profile before and after bariatric surgery.

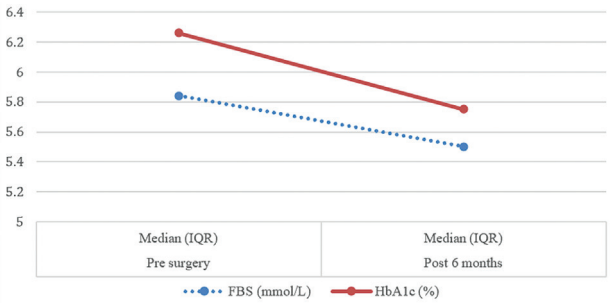

Figure 3. Graph showing comparison of glycemic index before and after bariatric surgery.

Table 2. Pre- and post-surgery results for anthropometric data, fasting lipid profile and glycemic metrics

Variables	Pre-surgery, N = 28	Post-surgery 6 months, N = 28	Mean difference	*p**
Weight in kg (SD)	123.4 (20.2)	99.3 (14.0)	24.1 (19.8 - 28.5)	<0.001
BMI in kg/m^2 (SD)	44.19 (7.29)	35.61 (5.50)	8.59 (7.08 - 10.09)	<0.001
Systolic BP in mmHg (SD)	141.2 (21.3)	121.4 (6.8)	19.8 (11.4 to 28.2)	<0.001
Diastolic BP in mmHg (SD)	86.6 (10.5)	74.4 (6.0)	12.2 (8.7 to 15.7)	< 0.001
Total Cholesterol in mmol/L (SD)	5.36 (1.15)	4.53 (0.76)	0.83 (0.51 to 1.16)	<0.001
Triglycerides in mmol/L (SD)	1.57 (0.59)	1.40 (0.34)	0.17 (0.04 to 0.31)	<0.001
LDL in mmol/L (SD)	3.55 (1.03)	2.70 (0.78)	0.85 (0.55 to 1.16)	<0.001
HDL in mmol/L (SD)	1.12 (0.2)	1.22 (0.16)	-0.09 (-0.16 to -0.03)	0.009
FBS in mmol/L (range)	5.84 (4.87-7.42)	5.50 (5.10-6.56)		0.119**
HbA1c in % (range)	6.26 (5.8-8.13)	5.75 (5.4-7.0)		0.001**
HbA1c in % (range) among subjects with diabetes, n = 9	7.7 (6.03-10.48)	6.70 (5.55-7.68)		0.009**
HbA1c in % (range) among subjects without diabetes, n = 19	5.80 (5.40-6.28)	5.5 (5.40-5.78)		0.018**
LDL in mmol/L (SD) among subjects with dyslipidemia, n = 15	3.67 (0.85)	3.00 (0.62)	0.54 (0.18-0.87)	<0.001
LDL in mmol/ (SD) among subjects without dyslipidemia, N = 13	3.41 (1.22)	2.35 (0.81)	1.05 (0.55-1.58)	0.002

Table 3. Distribution of subjects according to echocardiographic data before and after surgery

Variables	Pre-surgery (all patients), N = 28	Post-surgery 6 mos (all patients), N = 28	p *	Pre-surgery with normal DF, N = 13	Post-surgery 6 mos with normal DF, N = 13	Mean difference	Pre-surgery with abnormal DF, N = 15	Post-surgery 6 mos with abnormal DF, N = 15	Mean difference	*p**	Mean difference pre and post LSG, N = 19	Mean difference pre and post ReY	*p**
LAVI in ml/m^2 (range)	32.0 (27.3 - 36.0)	27.1 (25.0 - 33.0)	<0.001**	28 (25.6-31.5)	25 (24.5-28.05)	2.0 (0.6-2.2)	36 (33-42)	33 (27-34)	3.2 (2.0-8.9)	0.01**	2.00 (1-7)	3.0 (1.5-5.45)	0.905
Average E/e' (SD)	13.43 (2.71)	11.78 (2.48)	0.001	11.61 (2.29)	10.84 (2.51)	0.78 (-0.1-2.11)	14.93 (2.04)	12.36 (2.09)	2.57 (1.5-3.8)	0.04	1.19 (1.76)	2.73 (3.00)	0.105
Septal e' in cm/s (SD)	8.33 (1.70)	9.43 (1.59)	<0.001	9.25 (1.73)	10.06 (1.81)	0.046 (-1.32-1.73)	7.64 (1.19)	8.96 (1.15)	1.33 (-1.91-(-0.85))	0.137	-0.36 (2.5)	-1.4 (1.37)	0.263
Lateral e' in cm/s (SD)	11.37 (2.43)	12.03 (1.90)	0.002	13.21 (1.69)	13.32 (1.56)	-0.115 (-0.59 - 0.33)	9.99 (1.61)	11.06 (1.31)	-1.06 (-1.56 - -0.6)	0.009	-0.56 (0.89)	11.06 (1.31)	0.706
TR Vmax in m/s (SD)	2.53 (0.51)	2.36 (0.29)	0.012	2.20 (0.37)	2.23 (0.25)	-0.03 (-0.15 - 0.09)	2.84 (0.45)	2.49 (0.28)	0.34 (0.17-0.52)	0.003	2.84 (0.45)	-0.71 (1.19)	0.551
LV strain I in % (SD)	-21.2 (3.8)	-25.7 (3.8)	<0.001	-22.9 (3.33)	-26.84 (3.21)	3.85 (2.2-5.56)	-19.79 (3.77)	-25 (3.21)	5.21 (3.64-6.67)	0.24	0.13 (0.32)	0.22 (0.41)	0.79
LVEF in % (SD)	67.7 (7.6)	67.3 (5.3)	0.663	66.76 (6.16)	67.53 (3.54)	-0.77 (-3.0-1.64)	69.14 (8.79)	67.57 (6.49)	1.57 (-1.77-4.35)	0.255	-0.22 (4.77)	1.77 (6.18)	0.361
E wave in cm/s (SD)	59.2 (10.3)	66.6 (13.9)	0.001	64.39 (9.79)	71.62 (13.55)	-7.0 (-11.73 - -3.27)	55.5 (8.44)	63.07 (13.27)	-7.57 (-15.14 - -1.5)	0.907	-8.49 (10.49)	-5.0 (11.66)	0.439
E:A (SD)	1.08 (0.24)	1.17 (0.25)	0.060	1.11 (0.19)	1.14 (0.21)	-0.03 (-0.127 - 0.08)	1.07 (0.27)	1.23 (0.27)	-0.15 (-0.32 - 0.0017)	0.206	-0.07 (0.24)	-0.14 (0.28)	0.46

Abbreviations: LAVI=Left atrial volume index, TR=Tricuspid regurgitation, LVEF=Left ventricular ejection fraction
*Paired t-test; **Wilcoxon signed-rank test. Mean difference (MD) with 95% confidence interval was calculated for paired t-test.

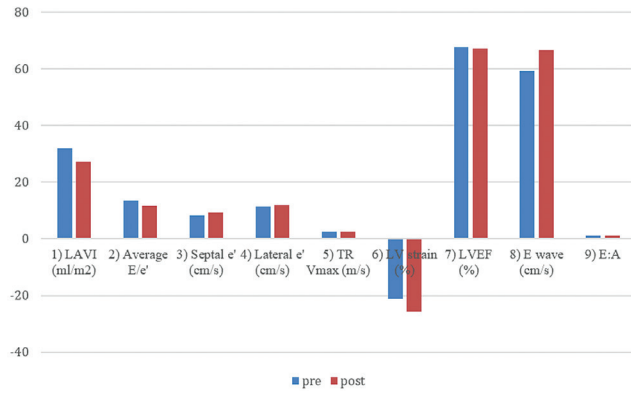

Figure 4. Graph showing comparison of echocardiographic parameters pre- and post-bariatric surgery.

Table 4. Changes in the grade of diastolic dysfunction post bariatric surgery

Grade (N,%)	Pre-surgery, N = 28	Post-surgery, N = 28	*p**
I	4 (14)	2 (7)	
II	7 (25)	0	
Indeterminate	4 (14)	0	
All	15 (54)	2 (7)	<0.05

Out of the 28 patients, 13 (46%) had normal diastolic function, while 15 (54%) had diastolic dysfunction at baseline. The distribution of the grading of their diastolic dysfunction were as follows: grade 1 (4 patients), grade 2 (7 patients) or indeterminate (4 patients). Post-bariatric surgery, echocardiographic data revealed that 26 (93%) had reverted to normal diastolic function while 2 (7%) remained to have diastolic dysfunction, as shown in Table 4.

Subgroup analysis on the effects of bariatric surgery on patients with pre-existing comorbidities

Among patients with and without diabetes, bariatric surgery improved HbA1c levels significantly, as seen in Table 2. Their LDL levels were likewise significantly reduced.

Subgroup analysis on the types of surgery on echocardiographic, glycemic and lipid profile

Nineteen patients (68%) underwent laparoscopic sleeve gastrectomy while nine (32%) underwent mini-gastric bypass with Roux-en-Y technique. The echocardiographic parameters, glycemic and lipid profiles were not significantly different between the two techniques as shown in Table 3.

Subgroup analysis on echocardiographic parameters between patients with normal diastolic function and established diastolic dysfunction

Thirteen (46%) patients had normal diastolic function at baseline while fifteen (54%) patients had diastolic dysfunction. Patients with pre-existing diastolic dysfunction had higher statistical improvements in terms of LAVI, average E/e', lateral e', and TR Vmax post-surgery.

DISCUSSION

Obesity is strongly associated with co-morbidities such as diabetes, hypertension, dyslipidemia and cardiovascular disease[27] which was seen in our study. As previously described, bariatric surgery has led to reductions in weight and associated illnesses.[28] Based on the results presented, there was a significant improvement in the left ventricular diastolic dysfunction after bariatric surgery. Our study also showed that patients with established diastolic dysfunction had improvements in their diastolic parameters (Table 5).

A literature review published in 2017 by Kindel et al., looked at bariatric surgery as one of the modalities to treat heart failure. The review showed that bariatric surgery reduced the risk of heart failure development and reversed abnormalities in cardiac mass, workload, and metabolism with improved diastolic function, potentially enhancing native cardiac systolic function.[29] An American Society of Echocardiography study in 2020 which looked at the impact of bariatric surgery on the echocardiographic features of cardiac remodeling and diastolic function concurred with our findings: patients with pre-existing diastolic dysfunction demonstrated improvements in diastolic function, driven by changes in TR velocity and medial E/e'.[30]

The advent of strain imaging echocardiography now offers a readily available and portable imaging tool that not only offers an objective characterization of myocardial dynamics but also allows for early detection of subclinical left ventricular dysfunction. Multiple studies have shown a direct correlation between obesity and GLS pattern.[31]

Table 5. Pre- and post-surgery results for anthropometric data, fasting lipid profile and glycemic metrics based on the type of surgery

Variables	Pre LSG, N = 19	Post LSG, N = 19	Mean difference	Pre ReY, N = 9	Post ReY, N = 9	Mean difference	*p**
Weight in kg (SD)	120 (18.91)	97.48 (15.54)	22.5 (18.94-27.14)	130.67 (22.05)	103.22 (9.77)	27.44 (18.26-37.09)	0.288
BMI in kg/m² (SD)	42.81 (6.35)	34.80 (5.57)	8.01 (6.73-9.43)	47.12 (8.61)	37.32 (5.23)	9.8 (6.45-13.17)	0.262
Systolic BP in mmHg (SD)	142.3 (22.45)	123 (6.94)	5.62 (5.14-6.03)	138.78 (19.72)	117.89 (5.44)	4.81 (3.97-5.7)	0.079
Diastolic BP in mmHg (SD)	85.94 (9.22)	73.21 (5.47)	12.74 (9.07-16.66)	88 (13.41)	77 (6.67)	11 (4.72-18.5)	0.676
LDL in mmol/L (SD)	3.77 (0.92)	2.89 (0.67)	0.78 (0.42-1.25)	3.08 (1.14)	2.3 (0.87)	0.78 (0.29-1.2)	0.985**
HbA1c in % (range)	6.22 (5.8-7.9)	5.7 (5.4-6.4)		6.7 (5.75-8.26)	6.1 (5.38-7.7)		0.594**

Abbreviations: SD = standard deviation, CI = confidence interval, BP = blood pressure, cm = centimeter, kg = kilogram, mmHg = millimeter mercury, LSG = Laparoscopic Sleeve Gastrectomy, ReY = Roux-en-Y gastric bypass
Data were expressed as mean (standard deviation) for normal distributions and median (first quartile-third quartile) for skewed distributions.
*Paired t-test; **Wilcoxon signed-rank test. Mean difference (MD) with 95% confidence interval was calculated for Paired t-test.

Our study showed that there was a statistically significant change in GLS pre and post-bariatric surgery. Similar to our findings, a prospective study by Lisa et al., showed that the LV longitudinal function largely recovered one year after bariatric surgery due to reduced afterload.[32]

In terms of the metabolic parameters, the lipid profiles of all the subjects significantly improved post-surgery. This was similar to the findings in a meta-analysis by Heffron et al., which looked at the changes in blood lipid levels of 47,779 subjects prior to and one year after bariatric surgery. Regardless of the surgery types (Roux-en-Y or sleeve gastrectomy), our study showed significant improvements in TC, TG, LDL and HDL.[33,34] We also observed an improvement in their glycemic control, with the HbA1c levels significantly decreased six months post-surgery. A finding also seen in a study done in Saudi Arabia by Ahmed et al.[35]

In summary, this study proves the benefit of bariatric surgery for patients with obesity, through the improvement of cardiac function and metabolic parameters. Although largely considered as a treatment of last resort for obesity, bariatric surgery may be a viable option in selected patients at high risk of developing cardiovascular events.

Limitations

The COVID-19 pandemic affected the recruitment of patients as many refused to participate. This is due to the risk of in-hospital infection, as our hospital is a COVID-19 treatment center. Another limitation is that we were unable to fully analyze the baseline echocardiograph results due to poor echo window and inadequate images saved on file.

CONCLUSION

Our study showed significant improvements in the lipid profile, glycemic control, and echocardiographic diastolic function parameters after bariatric surgery.

We also noted a significant improvement in the subjects' GLS parameters which warrants further studies in strain imaging.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
MHR: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft preparation; **MAR:** Software, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization; **SFM:** Validation, Resources, Supervision; **NRKNM:** Validation, Resources, Supervision; **HHCH:** Validation, Supervision, Project administration, Funding acquisition.

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Review Article

The Efficacy and Safety of Myo-inositol Supplementation for the Prevention of Gestational Diabetes Mellitus in Overweight and Obese Pregnant Women: A Systematic Review and Meta-Analysis

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Abstract

Background. Myo-inositol has emerged as one of the preventive therapies for the development of gestational diabetes mellitus in at-risk populations. This systematic review and meta-analysis was conducted to determine the efficacy and safety of myo-inositol in decreasing the incidence of gestational diabetes in overweight and obese pregnant women.

Methodology. This meta-analysis was conducted using the standard Cochrane methodology and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2020 guidelines. Inclusion criteria were randomized controlled trials (RCTs) that enrolled overweight and obese pregnant women and used myo-inositol supplementation. The primary outcome was the incidence of gestational diabetes mellitus at 24-28 weeks. Secondary outcomes included cesarean section rate, the incidence of pregnancy-induced hypertension, macrosomia and preterm delivery. Risk ratios (RRs) and 95% confidence intervals (CIs) were used for dichotomous data.

Results. Six RCTs were included. Compared to standard micronutrient supplementation, standard dose of myo-inositol (4 g) may reduce the incidence of GDM (RR 0.54; CI [0.30, 0.96]; n = 887 women), but the certainty of evidence is low to very low. With low-dose myo-inositol however, evidence is uncertain about its benefit on the incidence of gestational diabetes mellitus in overweight and obese women with RR 0.71; CI [0.14, 3.50]. No adverse effects were noted. For the secondary outcomes, standard dose myo-inositol appears to reduce the incidence of pregnancy-induced hypertension and preterm delivery, but the certainty of evidence is low to very low.

Conclusion. Current evidence is uncertain on the potential benefit of myo-inositol supplementation in overweight and obese pregnant women. While studies show that 4 g myo-inositol per day may decrease the incidence of GDM, pregnancy-induced hypertension and pre-term birth with no associated risk of serious adverse events, the certainty of evidence is low to very low. Future high-quality trials may provide more compelling evidence to support practice recommendations.

Key words: gestational diabetes, obesity, inositol phosphates

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as any level of glucose intolerance diagnosed for the first time during pregnancy.[1] Pregnancies complicated by GDM are at risk of both short-term and long-term consequences. Adverse fetal outcomes include large for gestational age infants and stillbirths,[2,3] while adverse maternal outcomes include the development of pre-eclampsia and gestational hypertension.[2,4]

Being overweight and obese increases the risk of gestational diabetes mellitus.[2,3] Adverse neonatal outcomes such as macrosomia have also been associated with elevated pre-pregnancy body mass index (BMI).[4] In Asians, a BMI ≥25 kg/m^2 was associated with an odds ratio (OR) of 3.27 for the development of GDM.[5]

Myo-inositol

Myo-inositol is an insulin-sensitizing agent naturally found in fruits, nuts and beans. Upon binding with its receptor (IR), insulin induces IRS-1 recruitment and activation. One of the principal IR/IRS targets, PI3K, then generates Phosphatidylinositol to activate PDK1 and subsequently PKB/Akt. These actions are involved in GLUT4 translocation and glycogen synthesis. In essence, myo-inositol acts as a secondary messenger that facilitates the transfer of glucose into the cell.[6]

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At the molecular level, insulin resistance is associated with a failure of insulin signaling, resulting in inadequate plasma membrane translocation of glucose transporter 4 (GLUT4). The increase in insulin sensitivity in patients who take myo-inositol may be an important intervention to prevent the development of GDM in high-risk women.[7]

Myo-inositol is a relatively cheap and widely available supplement that may be an effective strategy for GDM prevention in overweight/obese pregnant women. While studies have looked at the effect of myo-inositol supplementation in overweight and obese pregnant women, the sample sizes were not powered to detect differences in outcomes between groups.

OBJECTIVES

This review assessed if the supplementation of myo-inositol among pregnant women with a BMI ≥25 kg/m^2 is safe and effective in preventing GDM and other adverse maternal and neonatal outcomes. Specifically, we answered the following research question: Among pregnant women with BMI >25 kg/m^2, does supplementation with myo-inositol decrease the incidence of GDM, pregnancy-induced hypertension, cesarean section, preterm delivery and macrosomia?

METHODOLOGY

All published and unpublished randomized controlled trials assessing the effects of myo-inositol for the prevention of gestational diabetes mellitus among obese and overweight pregnant women were included. Case reports, observational studies and non-randomized trials were excluded.

We included trials that enrolled pregnant women classified as overweight or obese or whose body mass index is greater than or equal to 25 kg/m^2. Women already diagnosed with gestational diabetes mellitus and pregestational diabetes were excluded.

The intervention investigated was myo-inositol administered at any dose, alone or in combination, to prevent GDM and other adverse perinatal outcomes. Studies that compared the intervention with standard micronutrient supplementation alone or in combination were included.

The primary efficacy outcome was the incidence of Gestational Diabetes Mellitus (as defined by the IADPS Criteria). The primary safety outcome was the incidence of adverse effects. Secondary outcomes included incidence of pregnancy-induced hypertension and Cesarean section. For neonatal outcomes, the incidence of macrosomia and preterm birth were included.

Search terms included inositol, myo-inositol, gestational diabetes mellitus, GDM, obese and overweight. Randomized control trial was used as a filter. We searched the following databases from inception until March 3, 2022: The Cochrane Library, MEDLINE, Scopus, Google Scholar, MedRXIV, and Research Square. We also searched databases of unpublished, planned, and ongoing trials, including ClinicalTrials.gov (http://clinicaltrials.gov/), the EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/), and the World Health Organization (WHO) International Clinical Trials Registry Platform Search Portal (https://trialsearch.who.int/).

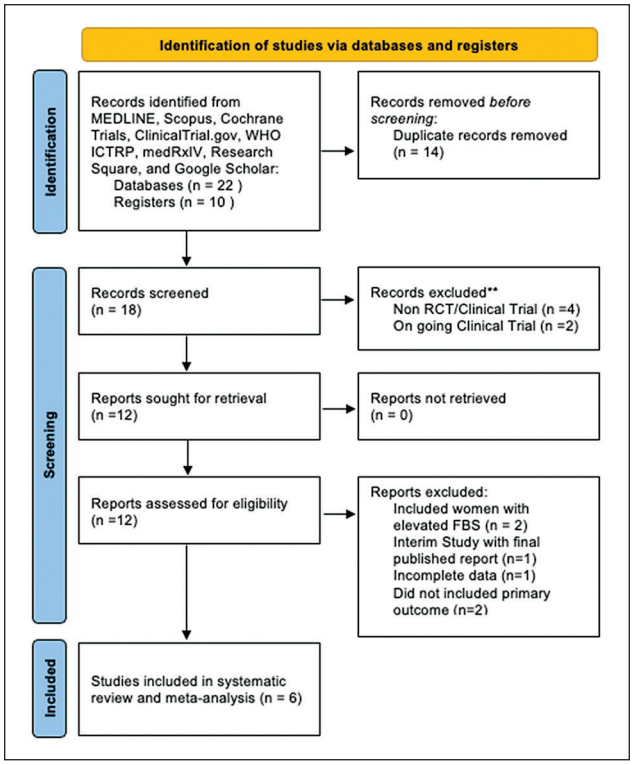

Figure 1. PRISMA Flowchart of Study Selection.

Data collection and analysis

Two review authors (HC, PF) independently scanned the title and abstract of every record retrieved to determine which studies should be assessed further. All potentially relevant articles were retrieved as full text and reviewed independently. Please refer to the adapted PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of study selection (Figure 1).

Data extraction and management

Data were extracted by the two review authors (HC, PF) using a data extraction form based on the Cochrane Pregnancy and Childbirth Group's data extraction form. Any disagreements were resolved by discussion. Review Manager (RevMan v 5.4.1) was used to encode all data. All data was encoded in Review Manager.

Assessment of risk of bias in included studies

Two review authors (HC, PF) assessed the risk of bias in each included study independently. Disagreements were resolved by discussion. We used the Cochrane

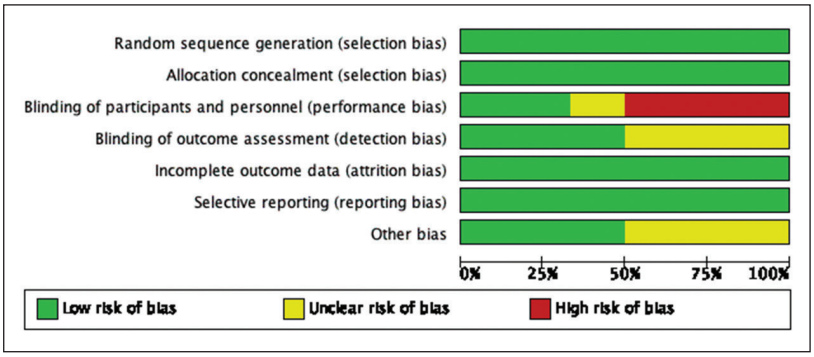

Figure 2. Risk of bias graph: review author's judgement about each risk of bias item presented as percentage across all included studies.

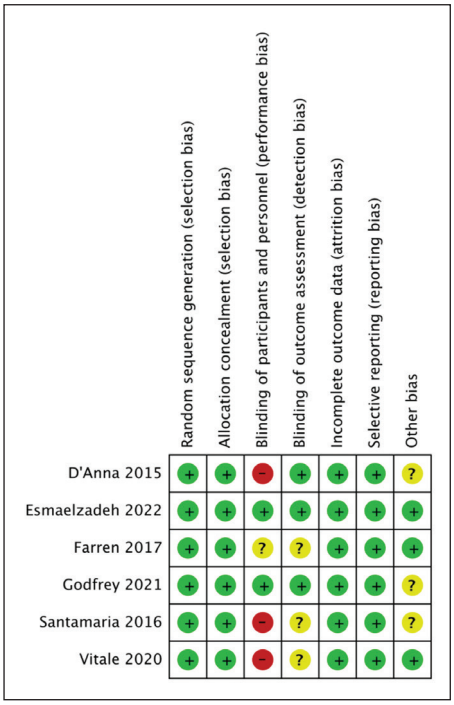

Figure 3. Risk of bias summary: Review authors' judgements about each risk of bias item for each included study.

Collaboration tool for the assessment of the risk of bias. We judged the risk of bias as 'low risk,' 'high risk' or 'unclear risk' and evaluated individual bias items as described in the Cochrane Handbook for Systematic Reviews of Interventions. The risk of bias within and across studies was presented graphically using RevMan (Figures 2 and 3).

Measures of treatment effect

Dichotomous data were expressed as risk ratios (RRs) with 95% confidence intervals (CIs).

Assessment of heterogeneity

Heterogeneity was identified by visually examining the forest plot and using a standard Chi-test^2 with a significance level of $\alpha = 0.05$. The I^2 statistic was used to assess the impact of heterogeneity on the meta-analysis; an I^2 statistic of 50% or more indicates a considerable level of inconsistency. Study results were not reported as pooled effects because of substantial clinical and methodological heterogeneity.

Data synthesis

Because of substantial clinical and methodological heterogeneity, a random-effects model was used to summarize data.

Subgroup analysis and investigation of heterogeneity

The authors did a subgroup analysis of standard dose (4 g) vs. low dose (≤2 g) of myo-inositol. Certainty of evidence was graded using GradePro, and discrepancies were settled through consensus.

RESULTS

Search strategy

We identified 32 reports; fourteen were duplicates, and six were excluded at the title and abstract stage. Of the twelve studies assessed for eligibility, only six were included. The study selection schematic diagram is shown in Figure 1.

Study characteristics

We included six published randomized controlled trials: D'Anna 2015, Santamaria 2016, Farren 2017, Vitale 2020, Godfrey 2021 and Esmaelzadeh 2022.[8–13] The baseline characteristics of the included studies are summarized in Table 1.

Duration of treatment with myo-inositol varied between studies. Most of the studies started upon recruitment at 10-16 weeks of gestation (AOG) and continued throughout the pregnancy.[8-11] In one study, it was given before conception until delivery.[12] In another study, myo-inositol was started upon recruitment at 12-14 weeks AOG until the 24th week of gestation.[13]

Effects of interventions

Primary outcomes

Incidence of gestational diabetes mellitus

For the mother, myo-inositol supplementation was associated with a reduction in the incidence of GDM in a

Table 1. General characteristics of the studies

References	Country and time of realization	Participants and main inclusion criteria	Intervention and timing	Intervention group	Control Group	Maternal health outcomes	Feto-neonatal health outcomes	Metabolic outcomes
D'Anna et al. 2015	Italy January 2011 - April 2014	220 obese pregnant women from Italy Eligibility criteria: pre-pregnancy BMI ≥30 kg/m^2, singleton gestation	Intervention: 4 g myo-inositol plus 400 mg folic acid daily (2 g myo-inositol + 200 mg folic acid orally twice a day), and nutritional and lifestyle counselling (n = 110) Duration of myo-inositol supplementation: from trial entry until the end of pregnancy	Group A: (n = 97) Age: 30.09 (18-44) BMI: 33.8 (30-46.9)	Group B: (n = 104) Age: 31.7 (19-43) BMI: 33.8 (30-46) 400 mg folic acid daily (200 mg folic acid orally twice a day), and nutritional and lifestyle counselling (n = 110)	GDM incidence Gestational hypertension Weight increase Adverse events CS rate	Preterm delivery Macrosomia Birth weight GA at birth Neonatal hypoglycemia NICU admission	OGTT – FBS, 1st hr, and 2nd hr
Santamaria et al. 2016	Italy Beginning of 2012 (36 months duration)	Overweight pregnant women Inclusion criteria were: (1) pre-pregnancy BMI 425 and 530 kg/m^2; (2) first trimester fasting plasma glucose <126 mg/dl and/or random glycemia <200 mg/dl; (3) single pregnancy; and (4) Caucasian ethnicity.	Treatment arm: 2000 mg myo-inositol + 200 mcg folic acid 2x/day Duration of myo-inositol supplementation: from trial entry until the end of pregnancy	Group A: (n = 95) Age: 32.1 (± 4.8) BMI: 26.9 (± 1.3)	Group B: (n = 102) Age: 32.7 (± 5.3) BMI: 27.1 (± 1.3) 400 mcg folic acid per day	GDM incidence Gestational hypertension Weight increase Adverse events CS rate	Preterm delivery Macrosomia Birth weight GA at birth Neonatal hypoglycemia NICU admission	OGTT – FBS, 1st hr, and 2nd hr
Farren et al. 2017	Ireland January 2014 - January 2016	240 Pregnant women with a family history of DM recruited at their first visit between 10 and 16 weeks' gestation. Eligibility Criteria: Women with a family history in a first-degree relative of diabetes, either type 1 or type 2, were eligible for inclusion.	1,100 mg myo-inositol + 27.6 mg D-chiro inositol + 400 mcg folic acid Duration: from enrollment throughout pregnancy	Group A: (n = 120) Age: 31.1 ± 5.1 BMI: 26 ± 5.3	Group B: (n = 120) Age: 31.5 ± 5 BMI: 26.2 ± 5.5 400 mcg of folic acid per day	GD incidence Gestational hypertension Adverse events CS rate	Preterm delivery Shoulder dystocia Macrosomia Birthweight GA at birth Neonatal hypoglycemia NICU admission	OGTT

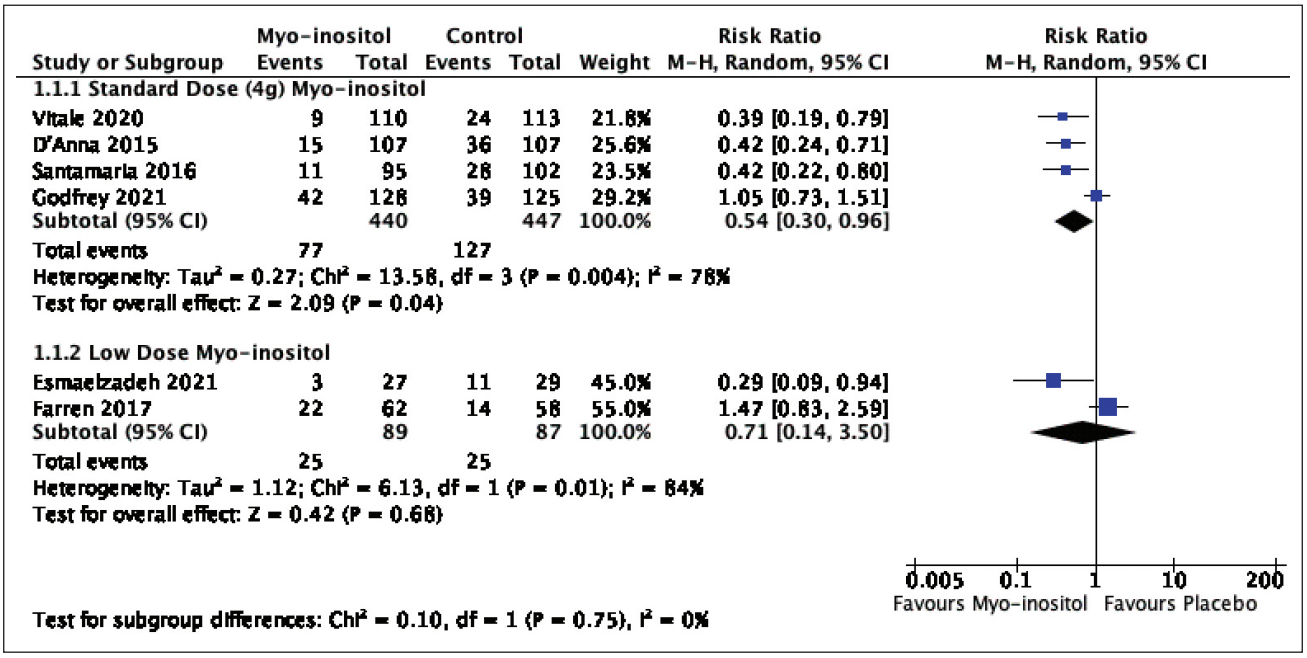

Figure 4. Forest plot of the effect of myo-inositol on the incidence of gestational diabetes mellitus in overweight and obese pregnant women.

Table 1. General characteristics of the studies *(continued)*

References	Country and time of realization	Participants and main inclusion criteria	Intervention and timing	Intervention group	Control Group	Maternal health outcomes	Feto-neonatal health outcomes	Metabolic outcomes
Godfrey et al. 2021	Randomized, double-blind, controlled trial Multisite: New Zealand, UK, and Singapore	Women 18-38 years old planning conception Eligibitlity Criteria: Aged 18–38 years, were planning to conceive within 6 months, and had future maternity care at the recruiting centers *included a sub-analysis of overweight and obese pregnant women using ethnic cut-offs: BMI >23 kg/m^2 for Asians including Chinese, Indians, Pakistani, Bangladeshi, Malay, mixed Asian; >25 kg/m^2 for non-Asians including White Caucasian, Polynesian, Black, mixed Asian-non-Asian	Intervention: additionally included myo-inositol 4 g/day, vitamin D 10 µg/day, riboflavin 1.8 mg/day, vitamin B6 2.6 mg/day, vitamin B12 5.2 µg/day, zinc 10 mg/day, and probiotics (Lactobacillus rhamnosus NCC 4007 [CGMCC 1.3724] and Bifidobacterium animalis subspecies lactis NCC 2818 [CNCM I-3446] Duration: from pre-conception throughout pregnancy	Group A: 128 No subgroup data regarding average age and BMI	Group B: 125 No subgroup data regarding average age and BMI Folic acid 400 µg/day, iron 12 mg/day, calcium 150 mg/day, iodine 150 µg/day, and β-carotene 720 µg/day	GDM incidence Adverse effects		OGTT
Vitale et al. 2020	Italy Beginning of 2016 and lasted 2 years	Overweight pregnant women Eligibility Criteria: pre-pregnancy BMI >25 and <30 kg/m^2, first-trimester fasting plasma glucose 126 mg/dl and/or random glycaemia <200 mg/dl, single pregnancy, and Caucasian ethnicity	Intervention: 2000 mg myo-inositol + 200 mcg folic acid 2x/day Intervention given from enrollment until 3 weeks postpartum	Group A: N = 110 Age: 27.18 ± 6.03 BMI: 27.00 ± 1.49	Group B: N = 113 Age: 27.95 ± 4.90 BMI: 26.68 ± 1.56 Control: Folic acid 200 mcg 2x/day	Incidence of GDM CS rate pregnancy-induced hypertension	macrosomia preterm delivery	change in lipid metabolism
Esmaelzadeh 2022	Iran From April 2018 - February 2020	Overweight pregnant women 12-14 weeks AOG Eligibility Criteria: Overweight pregnant women (BMI >25 kg/m^2)	Intervention: 2000 mg myo-inositol + 200 mcg folic acid a day Given from enrollment until 24 weeks AOG	Group A: n = 27 Age: 27.8 ± 4.2 BMI: 27.3 ± 1.8	Group B: n = 29 Age: 29.3 ± 4.4 BMI: 26.9 ± 1.9	GDM incidence Insulin therapy Weight gain Gestational hypertension CS rate	Preterm delivery Fetal macrosomia Shoulder dystocia NICU admissions RDS	OGTT Fasting insulin HOMA IR Total cholesterol trigyceride

dose-dependent manner in overweight and obese women. As seen in Figure 4, using the standard dose of 4 g, there appears to be a reduction in GDM (risk ratio 0.54, CI [0.30, 0.96]; n = 887). Using low-dose myo-inositol, the risk ratio is 0.71 with CI between [0.14, 3.50], crossing the line of no benefit. Thus, the evidence shows uncertain benefits of low-dose myo-inositol on the incidence of gestational diabetes mellitus in overweight and obese women.

There was significant heterogeneity in the studies, with I^2 of 78% and 82% for standard dose myo-inositol and low-dose myo-inositol, respectively. For standard dose myo-inositol, the heterogeneity is most likely due to differences in ethnicity, with Godfrey including mixed races, while D'Anna, Santamaria and Vitale had a predominantly Italian population.[8,9,11,12] The presence of other micronutrients with myo-inositol is also a notable difference between Godfrey and the studies done in Italy which may also explain the significant heterogeneity. For the low dose myo-inositol, I^2 was 82%; this may be explained by the difference in the study population, the duration of intervention and the addition of D-chiro-inositol.[10]

Using GRADEpro Guideline Development Tool, standard dose (4 g) myo-inositol may decrease the risk of gestational diabetes mellitus in overweight and obese pregnant women, while low dose myo-inositol has no effect on the incidence of GDM. Certainty of evidence is very low because of a high risk of performance bias, inconsistency and imprecision. The risk of GDM in women who received 4g myo-inositol is 15.3%, while for women in the control group, the risk is 28.4%.

Adverse events

All studies looked into the rate of adverse events with the intake of myo-inositol. In all studies, no significant adverse events were observed for both treatment and placebo groups.

Secondary outcomes

Incidence of cesarean section

Three trials reported on cesarean section rate with the intake of myo-inositol in obese and overweight pregnant women.[8,9,13] For standard dose myo-inositol, RR was 0.89

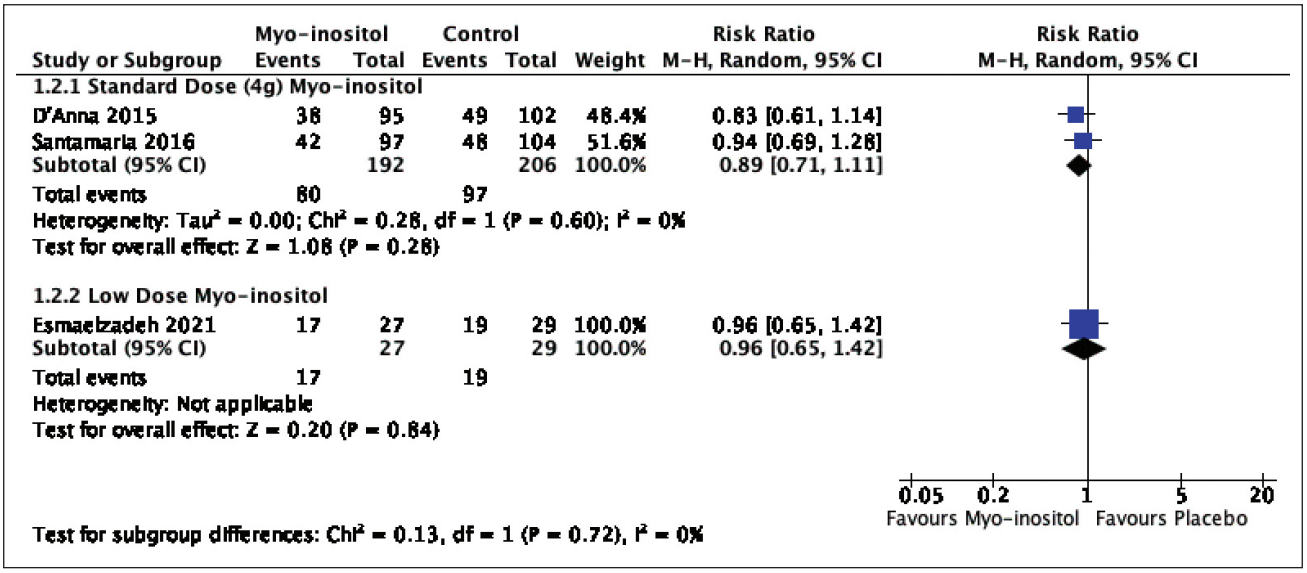

Figure 5. Forest plot of the effect of myo-inositol on the Cesarean Section rate in overweight and obese pregnant women.

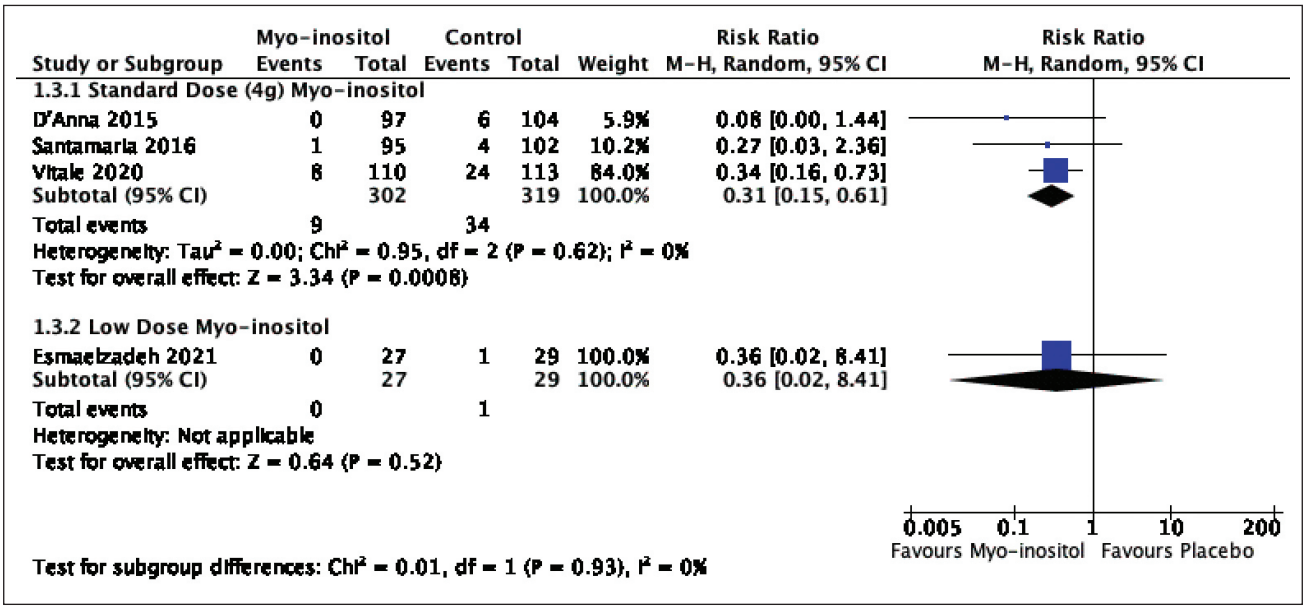

Figure 6. Forest plot of the effect of myo-inositol on the incidence of pregnancy-induced hypertension in overweight and obese pregnant women.

with 95% CI [0.71, 1.11], while for low dose myo-inositol, RR was 0.96 with 95% CI [0.65 to 1.42] as seen in Figure 5.

Based on the evidence, both 4 g myo-inositol and low-dose myo-inositol do not reduce the cesarean section rate in obese and overweight pregnant women. Evidence was of moderate to low certainty because of the serious risk of bias and imprecision.

Incidence of pregnancy-induced hypertension

Four studies as seen in Figure 6 examined the incidence of pregnancy-induced hypertension (PIH).[8,9,11,13] For the studies that used the standard dose of myo-inositol, the relative risk of PIH is 0.31 with 95% CI [0.15, 0.61], (3 trials, n = 621; random effects model). Standard dose myo-inositol seems to reduce the incidence of PIH, but this is based on very low certainty of evidence. The evidence of the benefit of 4 g myo-inositol in decreasing PIH was downgraded to very low because of the serious risk of bias in the studies included and the very serious imprecision in D'Anna and Santamaria, where the confidence intervals crossed the line of no benefit.

For low-dose myo-inositol, while the relative risk is 0.36, the 95% CI [0.02, 8.41] is too wide and crosses the line of no benefit. Low-dose myo-inositol does not decrease the incidence of PIH, based on low certainty of evidence due to very serious imprecision.

Incidence of pre-term birth

Three trials studied the incidence of preterm delivery in overweight and obese women who took myo-inositol.[8,9,13]

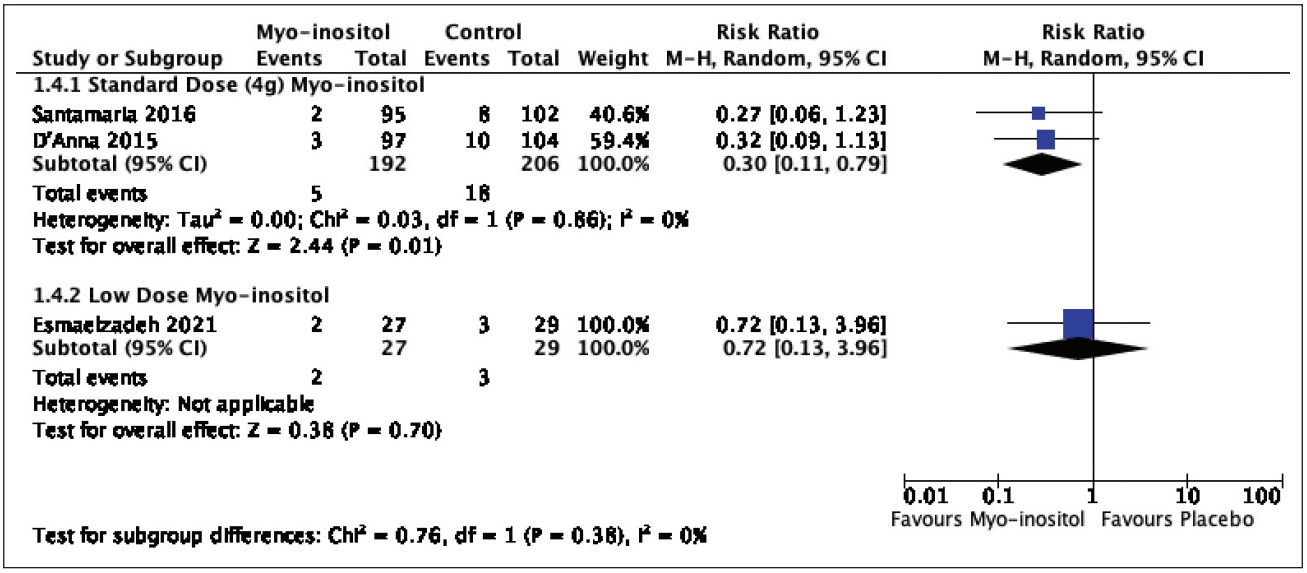

Figure 7. Forest plot of the effect of myo-inositol on the incidence of pre-term birth in overweight and obese pregnant women.

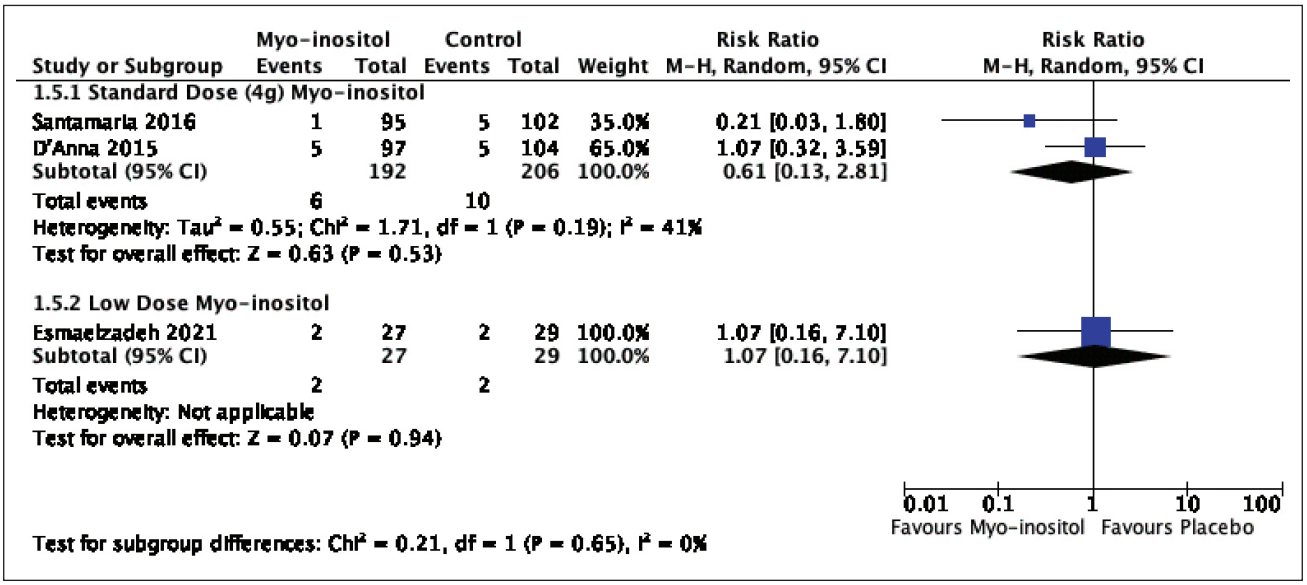

Figure 8. Forest plot of the effect of myo-inositol on the incidence of macrosomia in overweight and obese pregnant women.

In the subgroup of women who received standard dose myo-inositol, the RR was 0.30 with 95% CI [0.11, 0.61], (2 studies; n = 398), while for those given low dose myo-inositol, the RR was 0.72 with 95% CI [0.13, 3.96], (1 study, n = 56).[8,9,13] This is shown in Figure 7.

The evidence suggests that standard dose myo-inositol results in a slight reduction in the incidence of pre-term birth but certainty of evidence is low because of the serious risk of imprecision across the studies included. For low-dose myo-inositol, the evidence suggests that it does not decrease the risk of pre-term birth. The evidence is of low certainty because of the very wide confidence interval that crosses the line of no benefit in Esmaelzadeh.[13]

Incidence of macrosomia

One of the complications of gestational diabetes mellitus is the increased risk for macrosomia. Figure 8 shows the three studies that looked into the incidence of macrosomia in obese and pregnant women.[8,9,13]

In patients given standard dose myo-inositol, the RR was 0.61 with 95% CI [0.13, 2.81], (2 studies; n = 398). Since the confidence interval crossed the line of no benefit, the evidence suggests that myo-inositol does not decrease the incidence of macrosomia compared to standard micro-nutrient supplementation. The certainty of the evidence was downgraded to low because of the serious risk of performance bias, inconsistency across studies and imprecision in the pooled outcome. Low-dose myo-inositol, (RR 1.07 95% CI [0.16, 7.10], 1 study; n = 56), does not decrease the incidence of macrosomia in overweight and obese pregnant women. The certainty of the evidence was low due to the serious risk of bias and very wide confidence interval.[8,9,13]

DISCUSSION

Summary of main results

Evidence from six studies demonstrates a possible benefit of 4 g myo-inositol in reducing the incidence of gestational diabetes mellitus among overweight or obese pregnant women, but the evidence is uncertain. None of the studies reported serious adverse events from myo-inositol (Table 2).

For the secondary outcomes, there is a trend of reduction in the incidence of pre-term birth and incidence of pregnancy-induced hypertension in the standard dose (4 g) myo-inositol group, but the evidence is uncertain. The certainty of the evidence was downgraded because of the high risk of bias from the open-label design of the studies and the wide confidence intervals across the studies and in the pooled effects (Table 2).

Overall completeness and applicability of evidence

Participants in the included trials were pregnant women classified as overweight and obese or those with a BMI of 25 kg/m^2 or greater. These patients were at higher risk of developing GDM compared to women with normal BMI. Although one study included participants with different ethnicities, and another study conducted in the Middle East, the majority of the participants were Caucasians; hence applicability may be limited.

Certainty of evidence

Using GRADEpro, we determined the certainty of the current evidence for the incidence of GDM, caesarian section, pregnancy-induced hypertension, macrosomia and pre-term birth to be very low to low (Table 3).

The review results are based on six randomized controlled trials; three included trials were open-label in design and hence were assigned a high risk of bias in the parameter of blinding of participants and personnel. The certainty of the evidence was also downgraded because some results were inconsistent across studies. Due to the small number of patients and few observed events, the pooled results are less precise and confidence intervals are wide.

Potential biases in the review process

Communication was done through electronic mail with authors of studies when further information or clarification was needed. However, the literature search was limited to English-language articles.

CONCLUSIONS

Implications for practice

Supplementation with myo-inositol to reduce the risk of gestational diabetes mellitus among overweight or obese women is not currently in management guidelines. While evidence from this review demonstrated a possible benefit in the reduction of the incidence of GDM among overweight and obese pregnant women, certainty of the evidence is very low due to the high risk of bias (i.e., open-label design of many of the included studies), inconsistency of study results, and imprecision. The relatively small representation of other ethnicities with a high risk of gestational diabetes mellitus may also limit the applicability of current evidence.

In addition, the safety data evaluated was only for adverse events, and no long-term outcomes such as IQ, BMI or incidence of developmental delay among offspring of women given myo-inositol were reported.

Future high-quality clinical trials may provide more compelling evidence to support practice recommendations. At the moment, there is not enough evidence to support its clinical use for preventing GDM among overweight and obese women.

Implications for research

Applicability may be limited due to predominantly Caucasian participants in the included studies; hence future trials with representative ethnicities are recommended. Trials that reduce the risk of performance bias by ensuring the blinding of participants will also improve evidence quality.

Registration
This protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) on May 15, 2022 (CRD42022330250). Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022330250

Availability of data collection forms
Data collection forms and extracted data are available upon request to the corresponding author.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
PAF: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration; **HUC:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration.

Conflict of Interest
The authors have no conflicts of interest to disclose.

Funding Source
None.

Table 2. Summary of findings: Myo-inositol compared to standard micronutrient supplementation for prevention of GDM in overweight and obese pregnant women

Patient or population: prevention of GDM in overweight and obese pregnant women
Intervention: Myo-inositol
Comparison: standard micronutrient supplementation

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with standard micronutrient supplementation	Risk with Myo-inositol				
Incidence of Gestational Diabetes Mellitus						
Standard Dose (4 g) Myo-inositol	284 per 1,000	153 per 1,000 (85 to 273)	RR 0.54 (0.30 to 0.96)	887 (4 RCTs)	⨁◯◯◯ Very low[a,b,c]	Four grams myo-inositol may reduce/have little to no effect on incidence of gestational diabetes mellitus but the evidence is very uncertain.
Low Dose Myo-inositol	287 per 1,000	204 per 1,000 (40 to 1,000)	RR 0.71 (0.14 to 3.50)	176 (2 RCTs)	⨁◯◯◯ Very low[d,e,f]	The evidence is very uncertain about the effect of low dose myo-inositol on incidence of gestational diabetes mellitus.
Cesarean Section Rate						
Standard Dose (4 g) Myo-inositol	471 per 1,000	419 per 1,000 (334 to 523)	RR 0.89 (0.71 to 1.11)	398 (2 RCTs)	⨁⨁◯◯ Low[g,h]	The evidence suggests that 4 g myo-inositol does not reduce cesarean section rate.
Low Dose Myo-inositol	655 per 1,000	629 per 1,000 (426 to 930)	RR 0.96 (0.65 to 1.42)	56 (1 RCT)	⨁⨁⨁◯ Moderate[i]	Two grams myo-inositol probably does not reduce cesarean section rate
Incidence of Pregnancy Induced Hypertension						
Standard Dose (4 g) Myo-inositol	107 per 1,000	33 per 1,000 (16 to 65)	RR 0.31 (0.15 to 0.61)	621 (3 RCTs)	⨁◯◯◯ Very low[a,j]	Four grams myo-inositol may reduce/have little to no effect on pregnancy induced hypertension but the evidence is very uncertain.
Low Dose Myo-inositol	34 per 1,000	12 per 1,000 (1 to 290)	RR 0.36 (0.02 to 8.41)	56 (1 RCT)	⨁⨁◯◯ Low[k]	The evidence suggests that 2 g myo-inositol does not reduce pregnancy induced hypertension.
Incidence of Pre-term Birth						
Standard Dose (4 g) Myo-inositol	87 per 1,000	26 per 1,000 (10 to 69)	RR 0.30 (0.11 to 0.79)	398 (2 RCTs)	⨁⨁◯◯ Low[a,l]	The evidence suggests 4 g myo-inositol results in a slight reduction in incidence of pre-term birth.
Low Dose Myo-inositol	103 per 1,000	74 per 1,000 (13 to 410)	RR 0.72 (0.13 to 3.96)	56 (1 RCT)	⨁⨁◯◯ Low[m]	The evidence suggests that 2 g myo-inositol does not reduce incidence of pre-term birth.
Incidence of Macrosomia						
Standard Dose (4 g) Myo-inositol	49 per 1,000	30 per 1,000 (6 to 136)	RR 0.61 (0.13 to 2.81)	398 (2 RCTs)	⨁◯◯◯ Very low[a,i,n,o]	The evidence is very uncertain about the effect of 4 g myo-inositol on incidence of macrosomia.
Low Dose Myo-inositol	69 per 1,000	74 per 1,000 (11 to 490)	RR 1.07 (0.16 to 7.10)	56 (1 RCT)	⨁⨁◯◯ Low[p]	Two grams myo-inositol may result in little to no difference in incidence of macrosomia.

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

CI: confidence interval; RR: risk ratio

GRADE Working Group grades of evidence:
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations:
a. Very serious risk of bias because D'Anna 2015, Santamaria 2016, and Vitale 2020 were open-label studies.
b. Serious inconsistency, in Godfrey 2021, the intake of myo-inositol is of no clear benefit from placebo in GDM incidence with a RR 1.05 (95% CI = 0.73, 1.51) compared to the other 3 studies (D'Anna 2015, Santamaria 2016, and Vitale 2020) which all showed benefit.
c. Serious imprecision, the RR for developing GDM in the 4 g dose group is 0.54 but the 95% CI = 0.30, 0.96 is very wide.
d. Serious risk of bias, for Farren 2017, there was no mention of blinding of participants, personnel, and outcome assessors.
e. Serious inconsistency - Farren 2017 showed an increase in the incidence of GDM, while Esmaelzadeh 2022 showed some benefit.
f. Serious imprecision - the confidence interval for preventing GDM is very wide, RR 0.71 95% CI = 0.14, 3.50.
g. Very serious risk of bias because D'Ana 2015 and Santamaria 2016 are both open-label studies.
h. Serious imprecision - the confidence intervals of the individual studies and the pooled effects are wide and cross the line of no benefit.
i. Serious imprecision - the confidence interval is wide for Esmaelzadeh 2022.
j. Very serious imprecision - D'Anna 2015 and Santamaria 2016 have wide confidence intervals that crossed the no-effect line.
k. Serious imprecision - The Esmaelzadeh 2022 study has a very wide confidence interval for PIH (95% CI = 0.02, 8.41) which crosses the line of no effect.
l. Serious imprecision - The confidence interval for both D'Anna 2015 and Santamaria 2016 crossed the line of no benefit.
m. Very serious imprecision - The confidence interval for the Esmaelzadeh 2022 study in reducing the risk of pre-term birth is very wide, with 95% CI = 0.16, 7.1.
n. Serious inconsistency - Santamaria 2016 showed a trend toward benefit in terms of macrosomia, while D'Anna 2015 showed no benefit of giving myo-inositol for decreasing macrosomia. Furthermore, the pooled effects crossed the line of no benefit.
o. Very serious imprecision - there was a wide confidence interval on the effect of 4 g myo-inositol on the incidence of fetal macrosomia.
p. Very serious imprecision - For the Esmaelzadeh 2021 study, the confidence interval for the incidence of macrosomia was wide, with 95% CI = 0.16, 7.1.

Table 3. Myo-inositol compared to standard micronutrient supplementation for prevention of GDM in overweight and obese pregnant women

Certainty assessment							Summary of findings				
							Study event rates (%)			Anticipated absolute effects	
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	With standard micronutrient supplementation	With Myo-inositol	Relative effect (95% CI)	Risk with standard micronutrient supplementation	Risk difference with Myo-inositol
Incidence of Gestational Diabetes Mellitus - Standard Dose (4 g) Myo-inositol											
887 (4 RCTs)	very serious[a]	serious[b]	not serious	serious[c]	none	⨁◯◯◯ Very low	127/447 (28.4%)	77/440 (17.5%)	RR 0.54 (0.30 to 0.96)	284 per 1,000	131 fewer per 1,000 (from 199 fewer to 11 fewer)
Incidence of Gestational Diabetes Mellitus - Low Dose Myo-inositol											
176 (2 RCTs)	serious[d]	serious[e]	not serious	serious[f]	none	⨁◯◯◯ Very low	25/87 (28.7%)	25/89 (28.1%)	RR 0.71 (0.14 to 3.50)	287 per 1,000	83 fewer per 1,000 (from 247 fewer to 718 more)
Cesarean Section Rate - 4 g Myo-inositol											
398 (2 RCTs)	serious[g]	not serious	not serious	serious[h]	none	⨁⨁◯◯ Low	97/206 (47.1%)	80/192 (41.7%)	RR 0.89 (0.71 to 1.11)	471 per 1,000	52 fewer per 1,000 (from 137 fewer to 52 more)
Cesarean Section Rate - 2 g Myo-inositol											
56 (1 RCT)	not serious	not serious	not serious	serious[i]	none	⨁⨁⨁◯ Moderate	19/29 (65.5%)	17/27 (63.0%)	RR 0.96 (0.65 to 1.42)	655 per 1,000	26 fewer per 1,000 (from 229 fewer to 275 more)
Pregnancy Induced Hypertension - 4 g Myo-inositol											
621 (3 RCTs)	very serious[a]	not serious	not serious	very serious[j]	none	⨁◯◯◯ Very low	34/319 (10.7%)	9/302 (3.0%)	RR 0.31 (0.15 to 0.61)	107 per 1,000	74 fewer per 1,000 (from 91 fewer to 42 fewer)
Pregnancy Induced Hypertension - 2 g Myo-inositol											
56 (1 RCT)	not serious	not serious	not serious	very serious[k]	none	⨁⨁◯◯ Low	1/29 (3.4%)	0/27 (0.0%)	RR 0.36 (0.02 to 8.41)	34 per 1,000	22 fewer per 1,000 (from 34 fewer to 256 more)
Incidence of Pre-term Birth - 4 g Myo-inositol											
398 (2 RCTs)	serious[a]	not serious	not serious	serious[l]	none	⨁⨁◯◯ Low	18/206 (8.7%)	5/192 (2.6%)	RR 0.30 (0.11 to 0.79)	87 per 1,000	61 fewer per 1,000 (from 78 fewer to 18 fewer)
Incidence of Pre-term Birth - 2 g Myo-inositol											
56 (1 RCT)	not serious	not serious	not serious	very serious[m]	none	⨁⨁◯◯ Low	3/29 (10.3%)	2/27 (7.4%)	RR 0.72 (0.13 to 3.96)	103 per 1,000	29 fewer per 1,000 (from 90 fewer to 306 more)
Incidence of Macrosomia - 4 g Myo-inositol											
398 (2 RCTs)	serious[a]	very serious[n]	not serious	very serious[i,o]	none	⨁◯◯◯ Very low	10/206 (4.9%)	6/192 (3.1%)	RR 0.61 (0.13 to 2.81)	49 per 1,000	19 fewer per 1,000 (from 42 fewer to 88 more)
Incidence of Macrosomia - 2 g Myo-inositol											
56 (1 RCT)	not serious	not serious	not serious	very serious[p]	none	⨁⨁◯◯ Low	2/29 (6.9%)	2/27 (7.4%)	RR 1.07 (0.16 to 7.10)	69 per 1,000	5 more per 1,000 (from 58 fewer to 421 more)

CI: confidence interval; RR: risk ratio

Explanations

a. Very serious risk of bias because D'Anna 2015, Santamaria 2016, and Vitale 2020 were open-label studies.
b. Serious inconsistency, in Godfrey 2021, the intake of myo-inositol is of no clear benefit from placebo in GDM incidence with a RR 1.05 (95% CI = 0.73, 1.51) compared to the other 3 studies (D'Anna 2015, Santamaria 2016, and Vitale 2020) which all showed benefit.
c. Serious imprecision, the RR for developing GDM in the 4 g dose group is 0.54 but the 95% CI = 0.30, 0.96 is very wide.
d. Serious risk of bias, for Farren 2017, there was no mention of blinding of participants, personnel, and outcome assessors.
e. Serious inconsistency - Farren 2017 showed an increase in the incidence of GDM, while Esmaelzadeh 2022 showed some benefit.
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o. Very serious imprecision - there was a wide confidence interval on the effect of 4 g myo-inositol on the incidence of fetal macrosomia.
p. Very serious imprecision - For the Esmaelzadeh 2021 study, the confidence interval for the incidence of macrosomia was wide 95% CI = 0.16, 7.1

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REVIEW ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

Effect of Yoga and Walking on Glycemic Control for the Management of Type 2 Diabetes: A Systematic Review and Meta-analysis

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Abstract

Background. A daily habit of yogic practice or walking, along with an oral hypoglycemic agent (OHA) could be beneficial for better control of type 2 diabetes mellitus (T2DM). We conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) to find out the efficiency of yoga or walking on glycemic control in T2DM.

Methodology. The present systematic review and meta-analysis were completed according to the PRISMA guidelines. The risk of bias in included studies was evaluated, by using the revised Cochrane risk-of-bias tool for randomized trials. Meta-analysis was implemented using RevMan software. Forest plots were used to illustrate the study findings and meta-analysis results.

Results. Sixteen studies were included in this systematic review, where 1820 participants were allocated to one of the following interventions: yoga, walking, and without any regular exercise (control group). Participants were between 17–75 years of age. Compared to the control group, the yoga group had a significant reduction in fasting blood glucose (FBG) by 31.98 mg/dL (95% CI = –47.93 to –16.03), postprandial blood glucose (PPBG) by 25.59 mg/dL (95% CI = –44.00 to –7.18], glycosylated hemoglobin (HbAlc) by 0.73% (95% CI = –1.24 to -0.22), fasting insulin by 7.19 µIU/mL (95% CI = –12.10 to –2.28), and homeostatic model assessment for insulin resistance (HOMA-IR) by 3.87 (95% CI = –8.40 to -0.66). Compared to the control group, the walking group had a significant reduction in FBG by 12.37 mg/dL (95% CI = –20.06 to –4.68) and HbA1c by 0.35% (95% CI = –0.70 to –0.01). Compared to the walking group, the yoga group had a significant reduction in FBG by 12.07 mg/dL (95% CI = –24.34 to – 0.20), HbA1c by 0.20% (95% CI = –0.37 to –0.04), fasting insulin by 10.06 µIU/mL (95% CI = –23.84 to 3.71) and HOMA-IR by 5.97 (95% CI = –16.92 to 4.99).

Conclusions. Yoga or walking with OHA has positive effects on glycemic control. For the management of T2DM, yoga has relatively more significant effects on glycemic control than walking.

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Key words: yoga, walking, type 2 diabetes, glycemic control, insulin resistance

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder characterized by chronic hyperglycemia.[1] It is affected by a combination of two primary factors: defective insulin secretion of pancreatic β-cells and the inability of insulin-sensitive tissues to respond appropriately to insulin.[2] Poor glycemic control among T2DM patients is a major community health problem and is a significant risk factor for the advancement of diabetic complications. Glycemic control is the key healing objective for the prevention of organ damage and other health-related problems from diabetes. A rapid change in people's lifestyle in terms of physical inactivity collectively increases metabolic complications and gives rise to the problems related to T2DM.[3]

Yoga is an ancient pre-Vedic science and a way of life. Yoga originated in ancient India over 5000 years ago. It mainly aims to develop the psychophysiological health of an individual. The practice of yoga embraces moral observances *(Yama)*, self-disciplines *(Niyama)*, physical postures *(Asana)*, voluntarily controlled breathing *(Pranayama)*, Sensory withdrawal *(Pratyahara)*, Concentration *(Dharana)*, Meditation *(Dhyana)*, and self-realization *(Samadhi)* and certain philosophical principles.[4] Regular yogic practice

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with proper scientific dose is beneficial for controlling numerous lifestyle diseases, including type 2 diabetes.[5] Walking is a natural and primitive exercise pattern that an individual follows from childhood. It is the fundamental base of locomotion and good exercise for the whole body.[6]

A daily habit of yogic practice reduces mental and oxidative stress and is beneficial to attain glycemic control.[5,7] A growing body of evidence reports that regular physical activity like walking or yoga has a beneficial effect on metabolic activity by helping to promote better glycemic control.[7,8] Scientific research on walking suggests that walking is one of the safest cardiovascular activities that improves glycemic control and insulin sensitivity.[6,8]

Walking and yoga have an impact on glycemic control and insulin resistance for type 2 diabetes patients. The aim of this systematic review and meta-analysis was to pool all experimental results of randomized control trials (RCTs) to update and consolidate the evidence on the effect of yoga and walking on glycemic control in patients with T2DM.

METHODOLOGY

The present systematic review and meta-analysis was completed following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.[9]

Search strategies

Data were collected by searching the online databases PubMed, Scopus, Web of Science, BioMed Central, ClinicalTrials.gov, and International Clinical Trials Registry Platform (ICTRP) to find out appropriate RCTs. The following keywords were used: 'type 2 diabetes,' 'T2DM,' 'yoga,' 'walking,' 'yoga and Type 2 diabetes,' and 'walking and Type 2 diabetes,' which is illustrated in Table 1. Appropriate trials were limited to human subjects and only trials published with the full text in the last 10 years (2012 to 2022) and written in English were included in this study. The related studies acquired from the above databases were assembled and duplicates were removed; some inappropriate studies were further screened and excluded by reading the title, abstract, and selected manuscripts. After the final assessment, eligible articles were included in the systematic review and meta-analysis. The total selection process is illustrated in Figure 1.

Eligibility criteria

Inclusion criteria

The existing studies followed the PICOS criteria,[10] including:

1. (P) Participants: patients with type 2 diabetes mellitus with standard medication (OHA).
2. (I) Intervention: walking and yoga.
3. (C) Control: without any regular exercise.
4. (O) Outcomes: fasting blood glucose (FBG), postprandial blood glucose (PPBG) and glycosylated hemoglobin (HbAlc), fasting insulin level and homeostatic model assessment for insulin resistance (HOMA-IR).
5. (S) Study design: randomized controlled trials (RCT).

Exclusion criteria

1. Participants: adolescents with T2DM (under 17 years of age) and geriatric age groups (above 75 years of age); those with severe diseases or any severe illness; pregnancy; those who were participating in another physical exercise program at the same time.
2. Study design: articles that were not RCTs were not included in the study;
3. Review studies, duplicate studies, only abstracts, conference proceedings, editorials, book chapters, and commentaries were excluded.
4. Studies published before the year 2012 were excluded.

Risk of bias assessment

The risk of bias in included studies was evaluated by using the revised Cochrane risk-of-bias tool for randomized controlled trials (RoB-2)[11] which is illustrated in Table 5. According to this tool, the risk of bias in the study was assessed through five Domains. 1. Risk of bias arising from the randomization procedure; 2. Risk of bias due to deviances from the intended interventions (effect of assignment to intervention and adhering to intervention); 3. Risk of bias due to missing results data; 4. Risk of bias in the measurement of the outcome; and 5. Risk of bias in the selection of the reported outcome. The risk of bias is classified as "Low risk," "Some concerns," and "High risk".

Statistical analysis

Quantitative outcomes were collected from the included studies[3,6,12-25] for the statistical meta-analysis was performed by using RevMan statistical software (version 5.4.1). In order to pool the measures of treatment effect, a random effects model based on the inverse variance method was

Table 1. Articles identified according to search sequence and database used for the systematic review

Bibliographic databases↓	Search strategies			
	Yoga vs control on T2DM	Walking vs control on T2DM	Yoga vs walking on T2DM	Other exercises on T2DM
PubMed = 154	64	52	16	22
Scopus = 113	40	38	17	18
Web of Science = 99	28	29	18	24
BioMed Central = 79	28	29	13	9
ClinicalTrials.gov (United States National Library of Medicine) = 27	12	8	4	3
International Clinical Trials Registry Platform (ICTRP) = 26	6	8	0	12

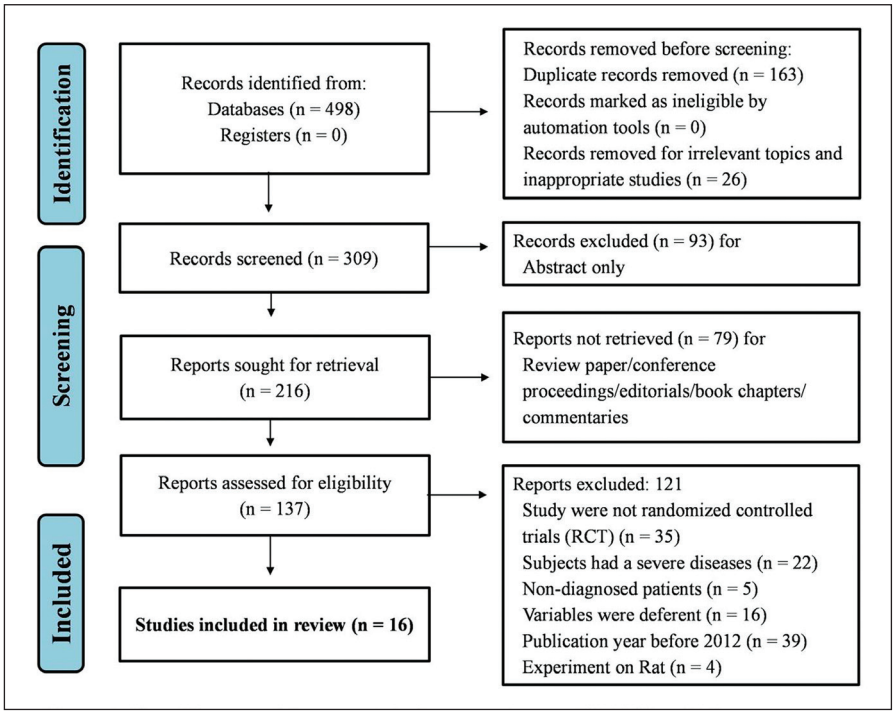

Figure 1. Flow diagram of the present study prepared as per PRISMA guidelines.

used. The effect size was calculated by taking the difference from mean and standard deviation (SD) of FBG, PPBG, HbA1c levels, fasting insulin, and HOMA-IR in the subjects before and after the intervention in both the experimental group and the control group. If the study failed to report this data, the effect size of the mean difference and SD difference was calculated by the following formula:[26,27]

$$\text{Mean difference} = \text{BaselineMean} - \text{FinalMean},$$

$$\text{SD difference} = \sqrt{\text{SD}^2\text{baseline} + \text{SD}^2\text{final} - (2 \times r \times \text{baselineSD} \times \text{finalSD})}$$

where r=0.7. Gowri et al., reported only the Median and interquartile range (upper and lower value) in their study so in that case from Median (m), First quartile (q1), and Third quartile (q3) sample Mean ($\bar{x}$) and SD was calculated by using this formula $x = \frac{q_1 + m + q_3}{3}$ and $SD = \frac{q_3 - q_1}{1.35}$.[28]

Mean difference and 95% confidence intervals were used as the summary statistic for the overall effect sizes. The I^2 statistic was used to test for heterogeneity of effect size among studies included in the meta-analysis. Forest plots were used to illustrate the study findings and meta-analysis results. FBG and PPBG are stated as mg/dL. HbA1c is stated as a percentage (%). Fasting insulin is stated as μIU/mL.

RESULTS

Study characteristics

After the removal of duplicates, screening of studies, and excluding some studies, 16 RCTs were finally included in this systematic review. Nine studies were included as a comparison of the yoga intervention group with the control group. They are summarized in Table 2. Seven studies were included as a comparison of the walking intervention group with the control group. They are summarized in Table 3. Six studies were included as a comparison of the yoga intervention group with the walking group. They are summarized in Table 4. Three studies were included as a comparison of yoga and walking with the control group.[6,12,13] These three studies were analyzed in three sub-groups (yoga vs control, walking vs control, and yoga vs walking). A total of 1820 participants (1054 males, 766 females) were included, and the age range of participants was 17–75 years.

In this review study, included articles used yoga interventions like Trikonasana, Paschimottanasana, Ardha-Matsyendrasana, Dhanurasana, Pawanmuktasana, Vakrasana, Bhujangasana, Anulom-vilom and Bhamri Pranayama, and relaxation techniques such as meditation, prayer, and Savasana. In the majority of the included studies, yoga interventions involved 30-60 minutes per day and five days per week (5 d/w) for twelve weeks (12 w). Subjects of every included study joined the yoga practice in the morning; these practices were facilitated by a yoga expert from the day of recruitment. Included studies had selected similar kinds of OHA: metformin and glimepiride.

This review study included articles that used walking interventions for 30-60 minutes, three days per week (3 d/w) for 8-12 weeks with moderate intensity (brisk walking) on a plane ground surface.

Risk of bias analysis

According to the criteria of the revised Cochrane risk-of-bias tool for randomized controlled trials that is illustrated in Table 5, eleven studies showed 'low risk of bias' because

these eleven studies were judged to be at low risk of bias for all domains. Four studies showed 'some concerns' as these studies were judged to raise some concerns in at least one domain for this effect, but not to be at high risk of bias for any domain. Two studies failed to maintain the criteria of RoB-2 for low risk of bias due to deviances from the intended interventions (intervention assignment).[12,14] One study failed to maintain the criteria of low risk of bias due to deviations from the intended interventions (adhering to intervention),[15] and one study failed to maintain the criteria of low risk of bias due to missing result data.[6] One study showed a 'high risk of bias'; this study was judged to be at high risk of bias in at least one domain for this outcome or the study is judged to have some concerns for multiple domains in a way that substantially lowers confidence in the result. This study was judged to be at 'high risk of bias' due to deviances from the intended interventions (intervention assignment), measurement of the outcome, and selection of the reported result.[24]

Effect of yoga on glycemic control

Glycemic control was determined by measuring FBG, PPBG, and HbA1c along with fasting insulin level and HOMA-IR. The effect on FBG was studied in nine studies (9 interventions, n = 1199)[3,6,12,13,16,17] included in the meta-analysis. Forest plots for FBG in Figure 2(I) show that there was a significant reduction in FBG in the yoga group in comparison to the control group. The pooled mean difference for FBG between the yoga group and control groups from random effects analysis was 31.98 mg/dL (95% CI = –47.93 to–16.03), and the statistical heterogeneity of the data as indicated by I^2 = 96% was statistically significant (p <0.00001). There were five studies (5 interventions, n = 899)[3,16-19] in which the effect of yoga on PPBG was studied. The pooled mean difference for PPBG between the yoga group and control groups from random effects analysis was 25.59 mg/dL (95% CI = –44.00 to –7.18; I^2 = 87%, p <0.00001) in Figure 2(II). HbA1c was assessed in four of the studies[3,16-18] included in the meta-analysis (4 interventions, n = 795). The pooled mean difference from

Table 2. Characteristics of included studies on yoga intervention and control group

Sl. No.	Authors and year	Participants (Recruited, age and sex)	Intervention (Type, intensity and duration)	Comparison condition	Outcomes	Study design
1	Gowri et al., 2022	Yoga – M/F 14/21, Age 54 ± 13 Control – M/F 23/12, Age 52.5 ± 11.2	Yoga 60 min/day, 2 days/week for 16 weeks	Control group with standard medication	FBG, PPBG, HbA1c, BMI, HOMA-IR, Lipids	RCT
2	Kaur et al., 2021	Yoga – M/F 19/72, Age 47.77 ± 9.59 Control – M/F 30/63, Age 49.24 ± 10.53	Yoga 60 min/day, 5 days/week for 12 weeks	Control group with standard medication	FBG, PPBG, HbA1c, BMI, WC, Lipids	RCT
3	Danasegaran et al., 2021	Yoga – M/F 40/0, Age 51.95 ± 6.17 Control – M/F 40/0, Age 51.48 ± 8.47	Yoga 40 min/day, 5 days/week for 12 weeks with medication	Control group with standard medication	FPG, BP, Insulin, BMI, Lipids	RCT
4	Viswanathan et al., 2021	Yoga – M/F 93/57, Age 50.8 ± 8.3 Control – M/F 103/47, Age 52.8 ± 7.0	Yoga 50 min/day, 5 days/week for 12 weeks	Control group with standard medication	FPG, PPPG, HbA1c, Lipids	RCT
5	Yuniartika et al., 2021	Yoga – M/F 7/11, Age 51.66 Control – M/F 8/10, Age 51.11	Yoga 60 min/day, 3 days/week for 12 weeks	Control group with standard medication	FBG, Lipids	RCT
6	Saberipour et al., 2020	Yoga – M/F 32/0, Age 48.25 ± 7.14 Control – M/F 33/0, Age 51.66 ± 11.06	Yoga 60 min/day, 3 days/week for 8 weeks	Control group with standard medication	FBG, Lipids, BP, BMI	RCT
7	Sharma et al., 2020	Yoga – M/F 32/20, Age 50.8 ± 8.3 Control – M/F 25/27, Age 52.8 ± 7.0	Yoga 40 min/day, 5 days/week for 24 weeks	Control group with standard medication	FBG, PPBG, HbA1c, Lipids, WHR	RCT
8	Keerthi et al., 2017	Yoga – M/F 31/29, Age 37.28 ± 6.21 Control – M/F 32/27, Age 36.72 ± 6.12	Yoga 38-45 min/day, 3 days/week for 12 weeks	Control group with standard medication	FPG, Fasting Insulin, HOMA-IR, QoL, IDRS	RCT
9	Kumpatla et al., 2015	Yoga – M/F 87/44, Age 41.0 ± 8.7 Control – M/F 71/39, Age 44.2 ± 7.4	Yoga 30 min/day, 7 days/week for 12 Weeks	Control group with standard medication	FPG, PPPG, HbA1c, BP, Lipids, BMI	RCT

FPG – Fasting Plasma Glucose; PPBG – Post-prandial Blood Glucose; HbA1c – Glycosylated hemoglobin; BMI – Body Mass Index; HOMA-IR – Homeostatic Model Assessment for Insulin Resistance; BMI – Body Mass Index; WC – Waist Circumference; PPPG – Post-prandial Plasma Glucose; FBG – Fasting Blood Glucose; RCT – Randomized Controlled Trial; WHR – Waist Hip Ratio, QoL – Quality of Life

Table 3. Characteristics of included studies of walking intervention and control group

Sl. No.	Authors and year	Participants (Recruited, age and sex)	Intervention (Type, intensity and duration)	Comparison condition	Outcomes	Study design
1	Leischik 2021	Waking – M/F 17/0, Age 60.4 ± 5.9 Control – M/F 16/0, Age 59.1 ± 8.5	Walking 40 min/day, 3 days/week for 12 weeks	Control group with standard medication	FPG, HbA1c, Lipids	RCT
2	Yuniartika et al., 2021	Walking – M/F 5/13, Age 61.33 Control – M/F 8/10, Age 51.11	Walking 30 min/day, 3 days/week for 12 weeks	Control group with standard medication	FBG, Lipids	RCT
3	Saberipour et al., 2020	Walking – M/F 33/0, Age 49.83 ± 9.58 Control – M/F 33/0, Age 51.66 ± 11.06	Walking 60 min/day, 3 days/week for 8 weeks	Control group with standard medication	FBG, Lipids, BP, BMI	RCT
4	Rafii et al., 2018	Waking – M/F 15/18, Age 53.18 ± 4.99 Control – M/F 14/20, Age 51.85 ± 7.83	Walking 30 min/day, 3 days/week for 8 weeks	Control group with standard medication	FBG, BMI	RCT
5	Akbarina et al., 2018	Waking – M/F 0/12, Age 61.92 ± 3.63 Control – M/F 0/12, Age 61.92 ± 3.63	Walking 45-60 min/day, 3 days/week for 8 weeks	Control group with standard medication	FBG, BMI, HbA1c, Lipids	RCT
6	Keerthi et al., 2017	Walking – M/F 30/28, Age 37.28 ± 6.21 Control – M/F 32/27, Age 36.72 ± 6.12	Walking 45 min/day, 3 days/week for 12 weeks	Control group with standard medication	FPG, Fasting Insulin, HOMA-IR, QoL, IDRS	RCT
7	Karstoft et al., 2013	Waking – M/F 4/8, Age 60.8 ± 2.2 Control – M/F 3/5, Age 57 ± 3.0	Walking 60 min/day, 5 days/week for 16 weeks	Control group with standard medication	FBG, Fasting Insulin, HbA1c, BP, Lipids.	RCT

FPG – Fasting Plasma Glucose; FBG – Fasting Blood Glucose; BP – Blood Pressure; BMI – Body Mass Index; HbA1c – Glycosylated hemoglobin; HOMA-IR – Homeostatic Model Assessment for Insulin Resistance; IDRS – Indian Diabetes Risk Score; RCT – Randomized Controlled Trial

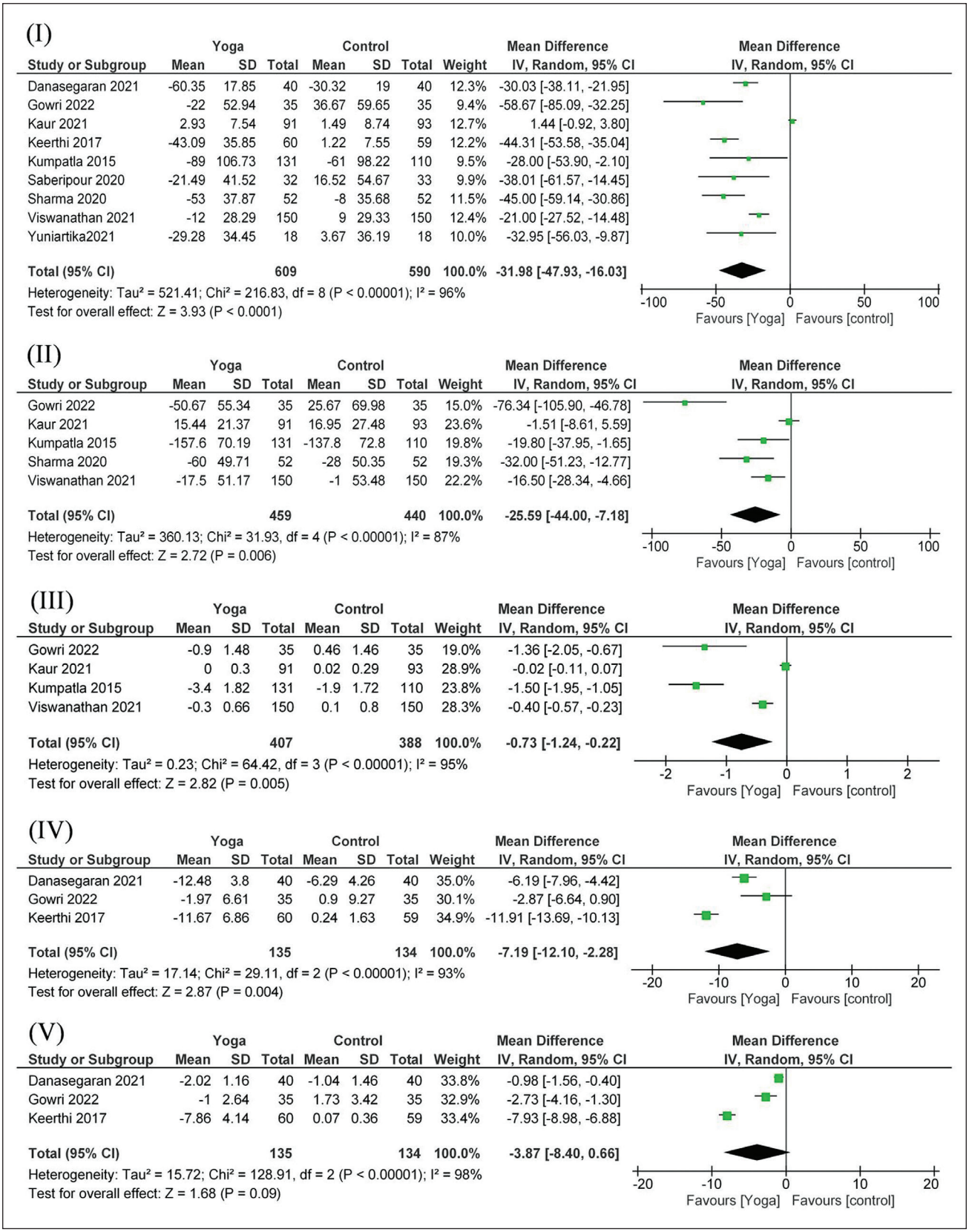

Figure 2. Forest plots presenting the effect of Yoga compared to Control group on **(I)** Fasting Blood Glucose, **(II)** Postprandial Blood Glucose, **(III)** Glycosylated Hemoglobin, **(IV)** Fasting Insulin and **(V)** Insulin Resistance.

Table 4. Characteristics of included studies on yoga intervention and walking intervention

Sl. No.	Authors and year	Participants (Recruited, age and sex)	Yoga (Type, intensity and duration)	Walking (Type, intensity and duration)	Outcomes	Study design
1	Yuniartika et al., 2021	Yoga – M/F 7/11, Age 51.66 Walking – M/F 5/13, Age 61.33	Yoga 60 min/day, 3 days/week for 12 weeks	Walking 30 min/day, 3 days/week for 12 weeks	FBG, Lipids	RCT
2	Gupta et al., 2020	Yoga – M/F 21/19, Age 51.1 ± 8.6 Walking – M/F 24/17, Age 50.2 ± 8.6	Yoga 45 min/day, 5 days/week for 16 weeks	Walking 30 min/day, 5 days/week for 16 weeks	FPG, SBP, DBP, HbA1c, Lipids, BMI, WC	RCT
3	Saberipour et al., 2020	Yoga – M/F 32/0, Age 48.25 ± 7.14 Walking – M/F 33/0, Age 49.83 ± 9.58	Yoga 60 min/day, 3 days/week for 8 weeks	Walking 60 min/day, 3 days/week for 8 weeks	FBG, SBP, DBP, Lipids, BMI	RCT
4	Singh et al., 2020	Yoga – M/F 41/60, Age 50.3 ± 9.1 Walking – M/F 49/50, Age 49.4 ± 8.7	Yoga 38-115 min/day, 5 days/week for 12 weeks	Walking 30 min/day, 5 days/week for 12 weeks	HbA1c, SSAI, STAI, BDI, ESE	RCT
5	Keerthi et al., 2017	Yoga – M/F 31/29, Age 37.28 ± 6.21 Walking – M/F 30/28, Age 37.28 ± 6.21	Yoga 38-45 min/day, 3 days/week for 12 weeks	Walking 45 min/day, 3 days/week for 12 weeks	FPG, Fasting Insulin, HOMA-IR, QoL, IDRS	RCT
6	McDermott et al., 2014	Yoga – M/F 9/12, Age 47.0 ± 9.7 Control – M/F 7/13, Age 47.2 ± 9.1	Yoga 75 min/day, 3-6 days/week for 8 weeks	Walking 30 min/day, 3-6 days/week for 8 weeks	FBG, PPBG, HbA1c, HOMA-IR, BP, Lipids	RCT

FBG – Fasting Blood Glucose; FPG – Fasting Plasma Glucose; SBP – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; HbA1c – Glycosylated hemoglobin; BMI – Body Mass Index; WC – Waist Circumference; SSAI – Spielberger's State Anxiety Inventory; STAI – Spielberger's Trait Anxiety Inventory; BDI – Beck Depression Inventory; ESE – Exercise Self-Efficacy; HOMA-IR – Homeostatic Model Assessment for Insulin Resistance; BP – Blood Pressure

Table 5. Risk of bias assessment of the included studies

Sl. No	Authors and year	Domain 1 (randomization process)	Domain 2 (assignment to intervention)	Domain 2 (adhering to intervention)	Domain 3 (missing outcome data)	Domain 4 (measurement of the outcome)	Domain 5 (selection of the reported result)	Overall risk of bias
1	Gowri et al., 2022	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
2	Kaur et al., 2021	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
3	Danasegaran et al., 2021	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
4	Viswanathan et al., 2021	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
5	Sharma et al., 2020	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
6	Kumpatla et al., 2015	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
7	Leischik 2021	Low risk	Low risk	Some concerns	Low risk	Low risk	Low risk	Some concerns
8	Rafii et al., 2018	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
9	Akbarina et al., 2018	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
10	Karstoft et al., 2013	Low risk	Some concerns	Low risk	Low risk	Low risk	Low risk	Some concerns
11	Yuniartikaet et al., 2021	Low risk	Low risk	Low risk	Some concerns	Low risk	Low risk	Some concerns
12	Gupta et al., 2020	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
13	Saberipour et al., 2020	Low risk	Some concerns	Low risk	Low risk	Low risk	Low risk	Some concerns
14	Singh et al., 2020	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
15	Keerthi et al., 2017	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
16	McDermott et al., 2014	Low risk	High risk	Low risk	Some concerns	High risk	High risk	High risk

random effects analysis was 0.73% (95% CI = –1.24 to -0.22; I^2 = 95%, p <0.00001) in Figure 2(III). There were three studies (3 interventions, n = 269)[3,13,20] for fasting insulin between the yoga group and control groups. The pooled mean difference from random effects analysis was 7.19 μIU/mL (95% CI = –12.10 to –2.28; I^2 = 93%, p <0.00001) in Figure 2(IV). HOMA-IR was assessed in 3 of the studies included in the meta-analysis (3 interventions, n = 269).[3,13,20] The pooled mean difference for HOMA-IR between the yoga group and control groups from random effects analysis was 3.87 (95% CI = –8.40 to -0.66) in Figure 2(V), and the statistical heterogeneity of the data as indicated by I^2 = 98% was significant (p <0.00001).

Effect of walking on glycemic control

Glycemic control was determined in the same way by measuring FBG, PPBG, and HbA1c along with fasting insulin and HOMA-IR. The effect on FBG was studied in 7 studies (7 interventions, n = 363)[6,12-15,21,22] included in the meta-analysis. There was a statistically significant reduction in FBG in the walking group in comparison to the control group. Forest plots for FBG Figure 3(I) show that there was a significant reduction in FBG in the walking group. The pooled mean difference for FBG between the walking group and control groups from random effects analysis was 12.37 mg/dL (95% CI = –20.06 to –4.68), and the statistical heterogeneity of the data as indicated by I^2 = 52% was statistically significant (p = 0.05). There were three studies (3 interventions, n = 77)[14,15,22] in which the effect of walking on HbA1c was studied. The pooled mean difference for HbA1c from random effects analysis was 0.35% (95% CI = –0.70 to –0.01; I^2 = 69%, p = 0.04) Figure 3(II). The change of HbA1c by 0.35% and FBG of 12.37 mg/dL in the walking group in comparison to the control group is statistically significant but may not be clinically significant.

Comparative effect of yoga and walking on glycemic control

The effect on FBG was studied in five studies (5 interventions, n = 335)[6,12,13,23,24] included in the meta-analysis. There was a statistically significant reduction in FBG in the yoga group in comparison to the walking group.

Forest plots for FBG in Figure 4(I) showed that there was a significant reduction in FBG in the yoga group. The pooled mean difference from random effects analysis was 12.07 mg/dL (95% CI = –24.34 to – 0.20; p = 0.03, I^2 = 62%). There were two studies (2 interventions, n = 278)[23,25] in which the effect of yoga and walking on HbA1c was studied. The pooled mean difference for HbA1c between the yoga group and walking group from random effects analysis was 0.20% (95% CI = –0.37 to –0.04; I^2 = 0%, p <0.90) in Figure 4(II). The effect on fasting insulin was studied in two studies (n = 156)[13,24] included in the meta-analysis. The pooled mean difference for fasting insulin between the yoga group and walking group from random effects analysis was 10.06 μIU/mL (95% CI = –23.84 to 3.71; I^2 = 98%, p <0.00001) in Figure 4(III). There were two studies (2 interventions, n = 159)[13,24] in which the effect of yoga and walking on HOMA-IR was studied. The pooled mean difference for HOMA-IR between the yoga group and walking group from random effects analysis was 5.97 (95% CI = –16.92 to 4.99; I^2 = 99%, p <0.00001) in Figure 4 (IV).

DISCUSSION

This meta-analysis observed either the effects of yoga or walking on glycemic control among patients with T2DM. Nine studies with 1197 adults (719 males, 478 females) comparing the yoga intervention to a control group were evaluated. Yoga interventions improved glycemic control by reducing HbA1c, FBG, PPBG, fasting insulin, and HOMA-IR compared to the control group. Seven studies with 365 adults (211 males, 154 females) comparing the walking intervention to a control group were evaluated. Walking interventions improved glycemic control by reducing HbA1c and FBG compared to the control group. Six studies with 541 adults (289 males, 252 females) comparing the yoga intervention to a walking intervention were evaluated. Yoga interventions improved glycemic control by reducing HbA1c, FBG, fasting insulin, and HOMA-IR compared to the walking intervention. Three studies were included comparing yoga and walking with control groups; these were analyzed in three sub-groups (yoga vs control, walking vs control, and yoga vs walking).[6,12,13]

Our results demonstrate a significant reduction in FBG (31.98 mg/dL), PPBG (25.59 mg/dL), HbA1c (0.73%), fasting insulin (7.19 μIU/mL), and HOMA-IR (3.87) in the yoga intervention compared to the control group (no exercise) in the pooled analysis. In the case of walking intervention compared to the control group (no exercise), the significant reduction of FBG was 12.37 mg/dL and HbA1c was 0.35% in the pooled analysis, but they did not evaluate the PPBG. Only Keerthi et al., evaluated fasting insulin and insulin resistance.[13] Similarly our results show a significant reduction in FBG (12.07 mg/dL), HbA1c (0.20%), fasting insulin (10.06 μIU/mL) and HOMA-IR (5.97) in the yoga intervention compared to the walking group in the pooled analysis. Kour et al., showed that after yoga intervention, the mean difference of glycemic control (FBG, PPBG, and HbA1c) decreased in a smaller amount than the control group in patients with type 2 diabetes mellitus.[16] McDermott showed that walking has more significant effects on FBG in comparison to yoga in type 2 diabetes mellitus patients.[24]

Viswanathan et al., revealed that there was a significant reduction in blood glucose levels and HbA1c in the yoga group as compared to the non-yoga group.[18] Kumpatla

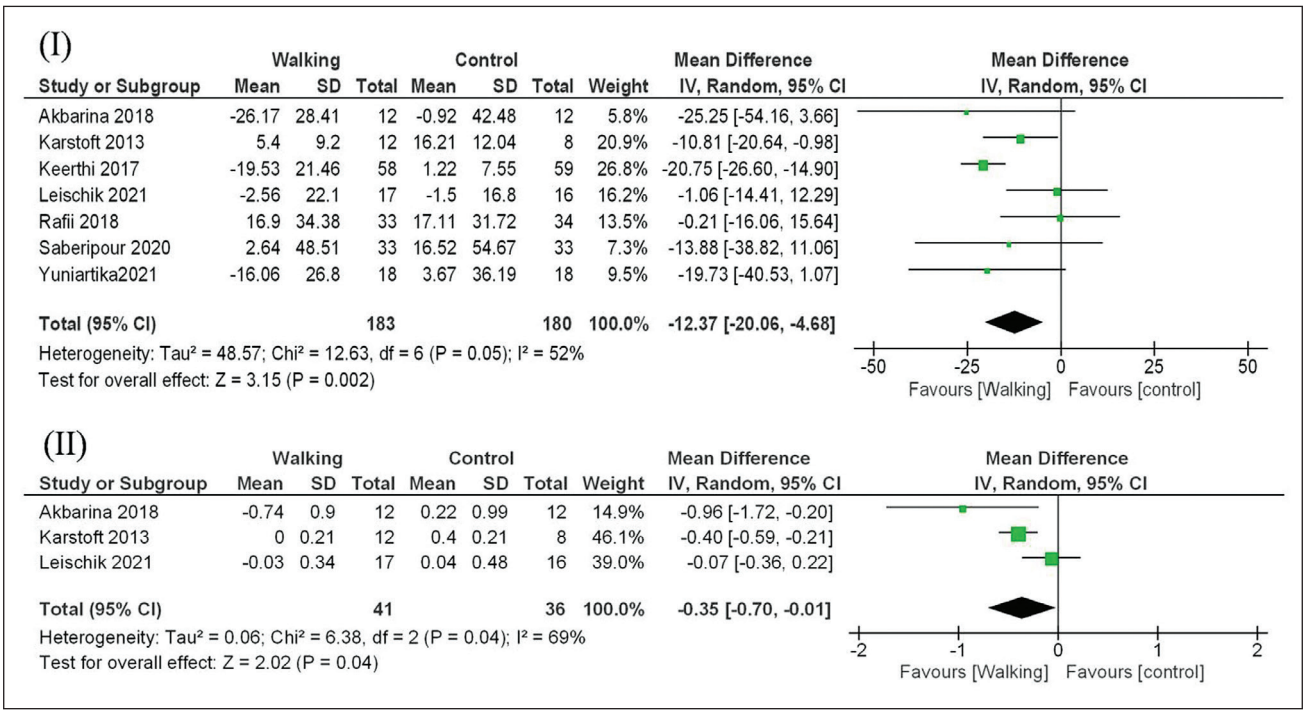

Figure 3. Forest plots presenting the effect of Walking compared to Control group on **(I)** Fasting Blood Glucose and **(II)** Glycosylated Hemoglobin.

et al., showed that the regular practice of yoga along with conventional medicines could be beneficial for better control of diabetes.[17] Saberipour et al., showed that yoga and walking had a positive effect on improving the laboratory indicators in men with type 2 diabetes, but yoga had more significant effects in diabetic patients as compared to walking.[12] Some studies exhibited a reduction in FBG, PPBG and HbA1c in the control group compared to the baseline and post-intervention due to the taking of oral hypoglycemic drugs (OHD),[15,17-20,22] but this change was not statistically significant. Diabetes is a psychosomatic disease related to both mind and body so psychoneuroendocrine and immune mechanisms are involved in the benefits of yoga on diabetes.[5]

Diabetes is a growing epidemic among lifestyle-associated cardiometabolic risk syndromes. It is accompanied by insulin resistance.[29] The idea of positive health was first introduced by Charaka, the father of the ancient Indian medical system called Ayurveda. He is the composer of the Ayurvedic foundational text, "Charaka Samhita." According to Charaka, body, mind, and soul are like a tripod.[30] In the Vasistha Samhita, we find two types of disease. One is mental (Adhija Vyadhi) and the other is physical (Anadhija Vyadhi).[31,32] Disease can germinate in either body or mind. Psychosomatic diseases are those that manifest in the mind and creep into the body, while in somatopsychic it is reversed. Yoga is a therapy that is a mind–body medicine.[33] Yoga as a part of Vedic philosophy that regards the human body as a combination of the mind, body, and soul.[34]

From this study, it may be recommended that Trikonasana, Paschimattanasana, Pawanmuktasana, Vakrasana,

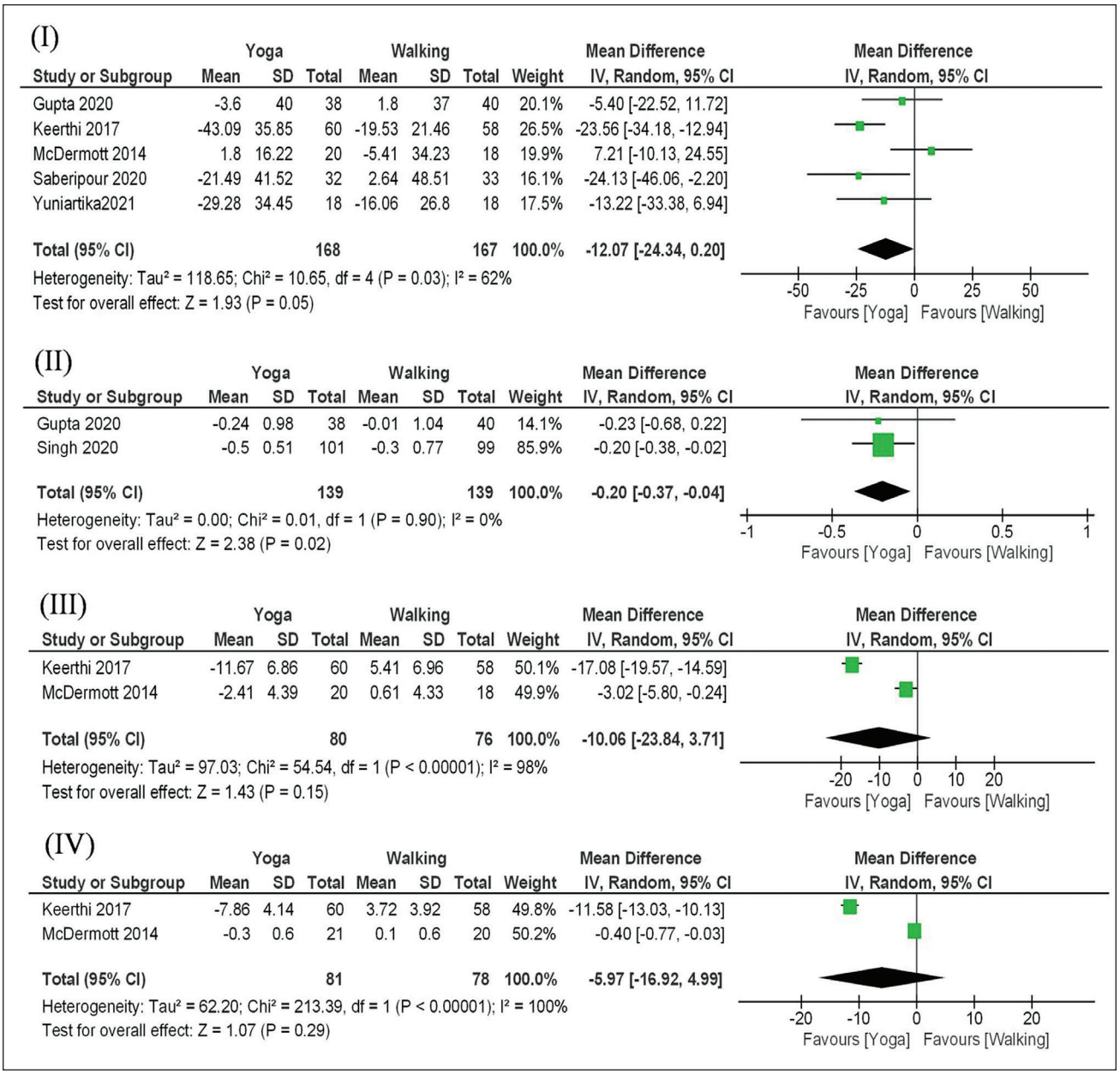

Figure 4. Forest plots presenting the effect of Yoga compared to Walking intervention on **(I)** Fasting Blood Glucose, **(II)** Glycosylated Hemoglobin, **(III)** Fasting Insulin and **(IV)** Insulin Resistance.

Bhujangasana, Ardha-Matseyendrasana, Dhanurasana, Sabasana, Kapalbhati, Anulom-Vilom and meditation for at least 45-60 minutes for five days per week can be beneficial for patients with diabetes. Walking five days per week and at least 45 minutes daily for people with diabetes can realize benefits to improve glycemic control. Additionally, concentration towards walking (Buddhist walking meditation) has a more favorable effect than the traditional walking program in patients with type 2 diabetes.[35] Future studies should emphasize the effects of different parameters of walking exercise on glycemic control of diabetes patients, such as walking frequency, walking time, and intensity.

CONCLUSIONS

In conclusion, this systematic review and meta-analysis provides evidence that either yoga or walking has positive effects on glycemic control and insulin resistance in comparison to the control group (no regular exercise) in patients with type 2 diabetes taking oral hypoglycemic agents. The change of HbA1c and FBG in the walking group compared to the control group is statistically significant but may not be clinically significant. Comparatively, yoga has more significant effects on glycemic control and insulin resistance in comparison to walking for the management of type 2 diabetes.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
BD: Methodology, Software, Validation, Formal analysis, Investigation, resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **SC:** Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration; **SSD:** Validation, Writing – review and editing, Supervision, Project administration; **MC:** Validation, Writing – review and editing, Supervision.

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CASE SERIES

Laron Syndrome: A Tale of Two Siblings

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Abstract

Primary growth hormone (GH) resistance or growth hormone insensitivity syndrome, also called Laron syndrome, is a hereditary disease caused by mutations in the GH receptor or in the post-receptor signaling pathway. This disorder is characterized by postnatal growth failure resembling GH deficiency. Differentiating the two conditions is necessary. We present the cases of two siblings, a 16-year-old female and a 9-year-old male, born from a consanguineous union. Both had normal birth weights with subsequent severe short stature and delayed teeth eruption, with no features suggestive of any systemic illness. Serum insulin-like growth factor 1 (IGF1) and insulin-like growth factor binding protein 3 (IGFBP3) were both low. Suspecting GH deficiency, provocative testing with clonidine was done revealing peak growth hormone >40 ng/mL in both patients. In view of low IGF1 and IGFBP3 and high GH on stimulation, IGF1 generation test was done for both siblings, with values supporting the diagnosis of GH insensitivity or Laron syndrome.

Key words: growth hormone insensitivity, Laron syndrome, short stature

INTRODUCTION

Laron syndrome, also known as growth hormone insensitivity, is an autosomal recessive disorder. It is caused by mutations, most commonly deletions, in the GH receptor gene or in the post-receptor signaling pathway, inducing low levels of IGF1.[1,2] Zvi Laron, an Israeli physician, first described this syndrome in 1966.[3] An estimated 350 individuals are affected by this syndrome globally, with a prevalence of 1 to 9 per 1,000,000. There are two large cohorts of patients with this syndrome living in Israel and Ecuador.[4]

Laron syndrome presents short stature, delayed dentition, delayed puberty, obesity and hypoglycemia. Genetic analysis is not always possible, particularly in resource-limited settings. As the disease resembles GH deficiency, it is imperative to differentiate the two for appropriate management. We present two cases of GH insensitivity from eastern India. Informed consent was obtained from their legal guardian (father).

CASE 1

A 16-year-old female born from a consanguineous marriage in Bengal, India, presented with severe short stature with unremarkable antenatal and perinatal history. Her birth weight was 2.8 kg. Birth length could not be recalled. At two to three years of age, her parents noticed no gain in height as compared to peers. She had a history of delayed tooth eruption. There was no family history of short stature or any personal history of systemic illness.

Examination revealed a height of 120.5 cm (<3rd centile) with height standard deviation score (SDS) -5.84 and body weight of 27.10 kg (<3rd centile) with weight SDS -2.41, according to the World Health Organization (WHO) 2006 and Indian Academy of Pediatrics (IAP) 2015 combined chart for girls. Mid-parental height was 141 cm. Upper-to-lower body segment ratio (US:LS) was 0.9. The patient had a prominent forehead and depressed nasal bridge indicative of facial dysmorphism, small hands and feet, crowded teeth with caries, a high-pitched voice, and thin, but not easily plucked scalp hair (Figure 1). Sexual Maturity Rating (SMR) was B4P2A+. No Turner's stigmata were present. Bone age was determined to be 13 years.

Complete hemogram, kidney function and liver function tests were normal. Hormonal assays revealed normal thyroid stimulating hormone (TSH) [1.6 mIU/mL, reference value (RV) 0.5 to 5 mIU/mL], free thyroxine (FT4) (0.9 ng/dL, RV 0.8 to 1.8 ng/dL), follicle stimulating hormone (FSH) (9.0 mIU/mL, RV 2 to 12 mIU/mL, follicular phase), luteinizing hormone (LH) (6.3 mIU/mlL, RV 1.0 to 18.0 mIU/mL, follicular phase) and cortisol (14 μg/dL, RV 5 to 25 μg/dL). Low basal IGF1 (34 ng/mL, RV 98 to 180 ng/mL)

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and IGFBP3 (504 ng/mL, RV 2,600 to 9,000 ng/mL) were also found.[5] Growth hormone stimulation test with clonidine revealed peak GH values more than 40 ng/mL (Table 1). Magnetic resonance imaging (MRI) of a the pituitary showed slight enlargement of the pituitary gland measuring 9 mm × 13.8 mm × 9.5 mm. (Figure 2). Ophthalmological evaluation revealed no blurring of vision, visual field defects or any other abnormalities.

In view of low IGF1 and IGFBP3 and high GH on stimulation, IGF1 generation test was done by injecting recombinant human growth hormone (hGH) at 33 μg/kg/day for 4 consecutive days. IGF1 level measured 12 hours after the last dose of hGH remained low (20 ng/mL), thus supporting the diagnosis of GH insensitivity or Laron syndrome. On Savage scoring, she fulfilled five out of seven parameters.[6] Genetic analysis was not performed due to financial limitations.

CASE 2

A 9-year-old male sibling of the female described in Case 1, born from the same parents, presented with severe short stature with unremarkable antenatal and perinatal history.

Figure 1. The patients **(A)** were a 16-year-old female (left, Case 1) and a 9-year old male (right, Case 2). Their heights were 120.5 cm (<3rd centile) **(B)** and 99.2 cm (<3rd centile) **(C)**, respectively, based on the WHO 2006 and IAP 2015 combined chart.

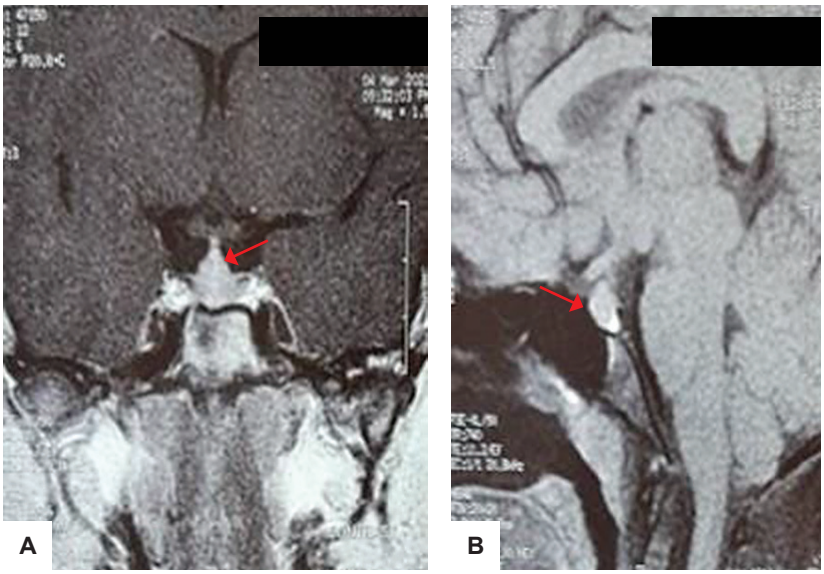

Figure 2. Magnetic resonance images of Case 1 showing an enlarged pituitary gland on coronal **(A)** and sagittal **(B)** views measuring 9 mm x 13.8 mm x 9.5 mm *(red arrow)*.

His birth weight was 3 kg. Birth length could not be recalled. At two to three years of his age, his parents noticed no height gain as compared to his peers. He also had a history of delayed tooth eruption.

Examination revealed a height of 99.2 cm (<3rd centile) with height SDS -5.04 and body weight of 14.20 kg (<3rd centile) with weight SDS -2.04, according to the WHO 2006 and IAP 2015 combined chart for boys. Mid-parental height was 154.5 cm. US: LS was 0.98. The patient had a prominent forehead and depressed nasal bridge suggestive of facial dysmorphism, small hands and feet, crowded teeth with caries, a high-pitched voice, and thin but not easily plucked scalp hair. SMR was prepubertal, with a stretched penile length of 3.4 cm indicative of micropenis. Bone age was determined to be at 3 years.

Complete hemogram, liver and kidney function tests were normal. Hormonal assays showed normal levels of TSH (2.9 mIU/mL, RV 0.5 to 5 mIU/mL), FT4 (1.2 ng/dL, RV 0.8 to 1.8 ng/dL), FSH (0.3 mIU/mL, RV 1 to 13 mIU/mL), LH (0.3 mIU/mL, RV <0.3 mIU/mL, prepubertal) and cortisol (12 μg/dL, 5 to 25 μg/dL). Basal IGF1 (15 ng/dL, RV 98 to 180 ng/mL) and IGFBP3 (398 ng/mL, RV 2,600 to 9,000 ng/mL) were both low.[5] Growth hormone stimulation test with clonidine revealed peak GH values of more than 40 ng/mL (Table 1). MRI of the pituitary gland was normal. An ophthalmological evaluation revealed no blurring of vision, visual field defects or any other abnormalities.

In view of low IGF1 and IGFBP3 and high GH on stimulation, the IGF1 generation test was done by injecting hGH at 33 μg/kg/day for 4 consecutive days. IGF1 level measured 12 hours after the last dose of hGH remained low (12 ng/mL), supporting the diagnosis of GH insensitivity or Laron syndrome. He fulfilled 5 out of 7 parameters on Savage scoring.[6] Genetic analysis was not performed due to financial limitations.

DISCUSSION

Laron syndrome or growth hormone insensitivity is a rare disorder. It is characterized by postnatal moderate to severe growth retardation in patients with normal birth weight and length. The height of patients varies between -4 to -10 SDS.[4] Final adult height in untreated patients ranges between 116 to 142 cm in males and 108 to 136 cm in females.[4] Features include prominent forehead; saddle nose; midfacial hypoplasia; thin, sparse and easily plucked hair; delayed dentition with overcrowding; high-pitched voice; micropenis; hypogonadism; hypoglycemia; obesity despite poor appetite; and a normal pituitary gland on imaging. Prior reports from India showed children with Laron syndrome may not be overweight.[7] None of our patients were obese or hypoglycemic. One of our patients is prepubertal with micropenis, while the other has delayed puberty. A comparative table between typical Laron syndrome and our cases is given in Table 2. The pituitary gland appears normal or hypoplastic on MRI in Laron syndrome, in contrast to the enlarged gland found in Case 1.[8] Although pituitary enlargement in Laron syndrome has not been reported in literature, this finding may be explained by pubertal enlargement or due to loss of feedback control of IGF1 to somatotrophs. The latter is similar to the feedback pathophysiology of adenoma in long-standing untreated primary hypothyroidism. This may be proven either by histopathology or by a decrease in size of the pituitary gland by IGF1 treatment. Due to GH resistance, patients with Laron syndrome have elevated GH but very low serum IGF1 that does not rise on exogenous administration of hGH.[9] These findings were also seen in our patients.

There are at least 10 different protocols of IGF1 generation tests for the diagnosis of GH insensitivity.[10] Some protocols use lower- (25 μg/kg/day) or high-dose (50 μg/kg/day) hGH over a period of four to seven days. The standard protocol uses recombinant GH at a dose of 33 μg/kg/day for seven days. Serum IGF1 is measured at baseline and 12 hours after the last dose of GH injection. Here, we used GH at a dose of 33 μg/kg/day for 4 consecutive days.

The only option for medical therapy in patients with Laron syndrome is recombinant human IGF1. The dose varies between 80 to 120 μg/kg twice daily administered subcutaneously.[11] Side effects include overgrowth of specific tissues, such as lymphatics, facial bones and kidneys; excessive increase of fat mass; hypoglycemia; hypokalemia;

Table 1. Results of growth hormone stimulation test with clonidine

Timing (min)	Growth hormone (ng/mL)	
	Case 1	Case 2
0	2.8	7.05
30	11.7	22.70
60	>40	>40
90	>40	>40
120	28.6	22.20

Table 2. Comparison of features of typical Laron syndrome and reported cases

Features	Typical Laron syndrome	Case 1	Case 2
Consanguinity	Usually present	Present	Present
Birth weight	Diminished or normal	Normal	Normal
Hypoglycemia	Usually present	Absent	Absent
Micropenis	Present	NA	Present
Height	Severely retarded	Severely retarded	Severely retarded
Bone age	Retarded	Retarded	Retarded
Dentition	Delayed	Delayed	Delayed
Midfacial hypoplasia	Present	Present	Present
Prominent forehead	Present	Present	Present
High-pitched voice	Present	Present	Present
IGF1, IGFBP3	Low	Low	Low
Stimulated GH levels	High	High	High
Pituitary imaging	Normal	Enlarged	Normal

water retention; and hypercalciuria. Non-availability of IGF-1 in India is a barrier to the treatment of children with Laron syndrome. However, even for untreated patients, normal life expectancy has been recorded up to 70 years in studies by Laron as well as by Rosenbloom in Ecuador.[4,12]

CONCLUSION

We have described two siblings with Laron syndrome who were referred for evaluation of short stature. To diagnose Laron syndrome, a high index of clinical suspicion is required in evaluating children with short stature with features of GH deficiency, high GH and low IGF1. Because recombinant IGF1 is not readily available, many patients are lost to follow up.

LEARNING POINTS

Laron syndrome is a rare but important cause of severe short stature, having clinical features similar to GH deficiency.

A high index of clinical suspicion is required for the diagnosis of Laron syndrome or growth hormone insensitivity in a child with severe short stature having high GH but low IGF1 and IGFBP3.

In Laron syndrome, the pituitary gland may be enlarged due to feedback stimulation secondary to GH resistance.

Ethical Considerations
Parental consent was obtained before submission of the manuscript.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
ND: Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization; **SST:** Conceptualization, Methodology, Resources, Writing – original draft preparation, Writing – review and editing, Visualization; **SS:** Methodology, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization; **PMG:** Conceptualization, Investigation, Writing – review and editing, Supervision, Project administration; **DKH:** Writing – review and editing; **SG:** Supervision, Project administration; **AB:** Resources, Supervision, Project administration; **NS:** Supervision, Project administration.

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Journal of the ASEAN Federation of Endocrine Societies

CASE REPORT

Corneal Confocal Microscopy Identifies Structural Small Fibre Abnormalities in an Adolescent with Type 1 Diabetes and Impaired Awareness of Hypoglycaemia

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Abstract

Impaired awareness of hypoglycaemia (IAH) is present in around 25-40% of individuals with type 1 diabetes mellitus (T1DM). Herein, we present a case of an adolescent with T1DM and IAH who had worse corneal nerve parameters compared to a T1DM adolescent without IAH. Small fibre abnormalities detected by corneal confocal microscopy in an objective easy-to-perform non-invasive test might be a surrogate indicator of underlying autonomic dysfunction in T1DM and IAH.

Key words: corneal confocal microscopy, small fibre, autonomic neuropathy, hypoglycaemia, T1DM

CASE

A 16-year-old adolescent Indian male with type-1 diabetes (T1DM), diagnosed 11 years ago, presented with a one-month history of intermittent episodes of confusion with documented hypoglycaemia. He was on a basal-bolus insulin regimen requiring 26 units/day. His Clarke score was 4, suggestive of impaired awareness of hypoglycaemia (IAH). He had mild tingling and numbness of the extremities with normal monofilament and vibration perception thresholds, suggestive of minimal evidence of clinical neuropathy (Toronto clinical neuropathy score [TCNS]=2). Warm detection thresholds were mildly abnormal. He had no retinopathy. His resting pulse rate was 104 beats/min. Laboratory parameters were: HbA1c 9.3% (78 mmol/mol), creatinine 1.0 mg/dl, urine albumin-creatinine ratio 124 μg/g creatinine. *In vivo* corneal confocal microscopy (CCM) was performed on the patient, an age-matched T1DM child without IAH (TCNS=1, HbA1c 9%, T1DM duration 10 years), and a healthy control to explore the underlying pathophysiological defects. The following parameters were used in the assessment of corneal nerve pathology: (i) corneal nerve fibre density (CNFD), (ii) corneal nerve fibre length (CNFL), (iii) corneal nerve branch density (CNBD).[1]

Figure 1 essentially indicates the presence of poor small nerve fibre morphology i.e., reduced CNFD, CNBD and CNFL in a T1DM child without IAH compared to an age-matched healthy control. When compared to the T1DM child without IAH, the images of our patient with IAH reveals poorer corneal nerve parameters, in particular reduced CNBD and CNFL.

DISCUSSION

Approximately 25–40% of individuals with T1DM have IAH, the hallmark of which is the attenuation of counter-regulatory sympathetic symptoms such as tremors, palpitations, and anxiety, and impaired neuro-hormonal response to hypoglycaemia.[2] Autonomic dysfunction, which includes cardiovascular autonomic neuropathy (CAN), contributes directly to IAH.[2] Cardiovascular reflex tests are considered the gold-standard method for confirming CAN. However, these battery of tests are often difficult to perform in routine clinical practice.[3] Abnormalities in the CCM parameters in diabetes have previously been shown to precede clinical neuropathic deficits and neurophysiological abnormalities of large fibres.[4,5] CCM can objectively detect early corneal nerve fibre damage in T1DM children even in the absence of clinical neuropathy, retinopathy or microalbuminuria.[6] CCM has also been proven to be useful in the assessment of CAN in T1DM.[7] However, the potential role of CCM in an individual with T1DM and IAH has not yet been previously explored. Postganglionic autonomic nerve fibres in both sympathetic and parasympathetic nervous system are unmyelinated small fibres, as are C-sensory fibres that are present in corneal sub-basal plexus. Herein, we demonstrate that the changes in CCM in a patient

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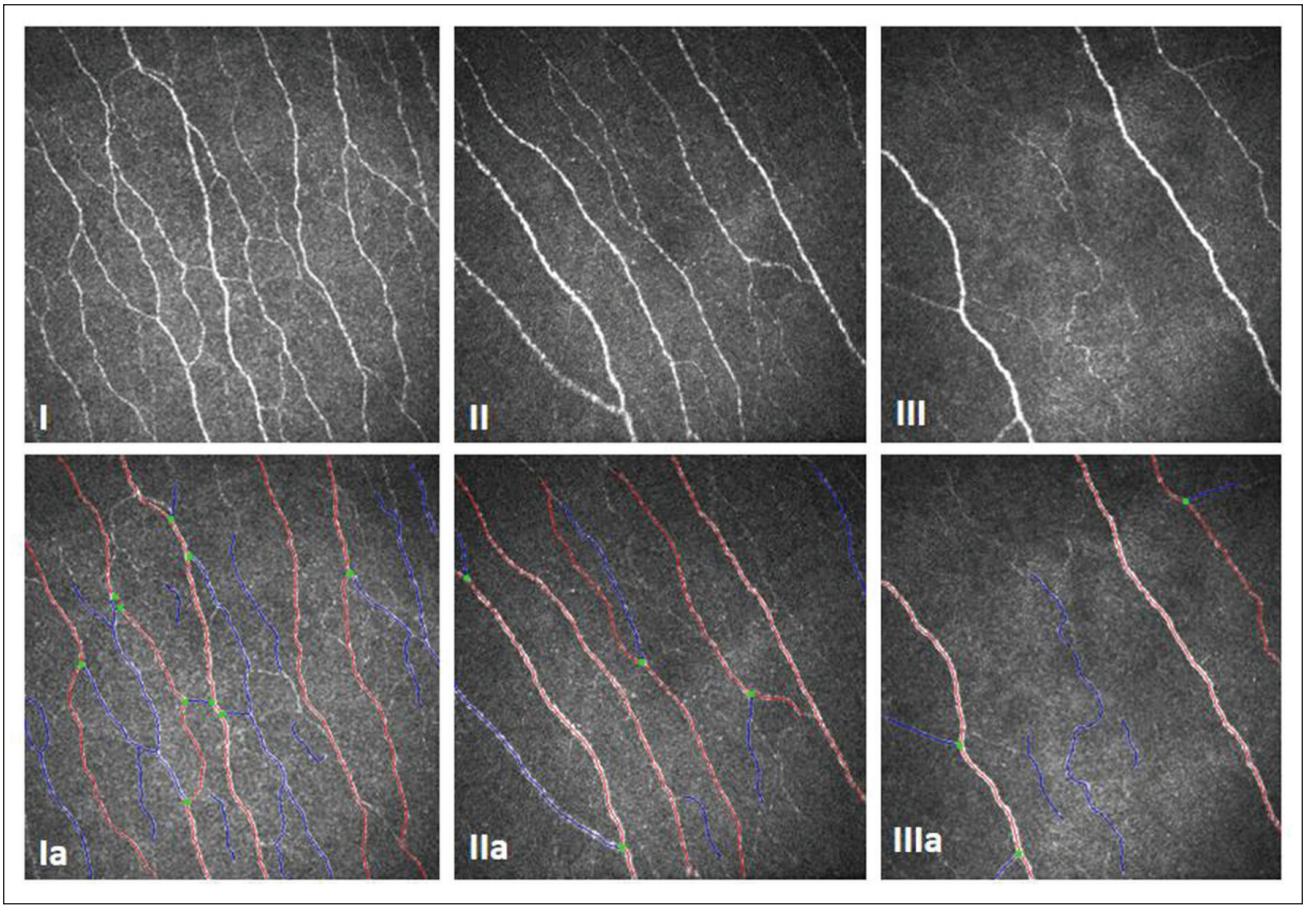

Figure 1. ***I and Ia: Images of a healthy 16-year-old adolescent.*** Original images of corneal sub-basal nerve plexus (I); Analysed images using CCMetrics software *(red: fiber; blue: branch; green: branch point)* showing normal corneal nerve fibre parameters *(fibre density: CNFD; branch density: CNBD; fibre length: CNFL)* (CNFD 31.2/mm^2, CNBD 62.5/mm^2, CNFL 21.1 mm/mm^2) (Ia); ***II and IIa: CCM images of 15-year-old boy with HbA1c 9% and T1DM duration of 10 years.*** Original images of corneal sub-basal nerve plexus (II); Analysed images showing reduced corneal nerve parameters (CNFD 18.7/mm^2, CNBD 38.7/mm^2, CNFL 16.0 mm/mm^2) (IIa); ***III and IIIa: CCM images in our patient*** *(described in case report)*. Original images of corneal sub-basal nerve plexus (III); Analysed images showing poor corneal nerve morphology in our patient (CNFD 16.8/mm^2, CNBD 28.7/mm^2, CNFL 11.0 mm/mm^2) (IIIa).

with IAH could prove to be an easy-to-perform and non-invasive surrogate test for the detection of underlying small nerve fibre abnormalities.

CONCLUSION

The present report highlights the potential clinical utility of CCM in detecting early small fibre abnormalities in T1DM and IAH. In the future, it would be interesting to perform longitudinal studies to evaluate if changes in CCM precede IAH or can predict the future risk of IAH in individuals with T1DM.

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Ethical Consideration
Patient consent was obtained before submission of the manuscript.

Statement of Authorship
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CRediT Author Statement
MB: Software, Data Curation, Writing – original draft preparation; **PM:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing – review and editing, Visualization, Supervision; **MB:** Software, Data Curation; **SG:** Conceptualization, Methodology, Software, Visualization, Formal analysis, Investigation, Resources, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition.

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Case Report

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Clinical Features of Unrecognized Congenital Adrenal Hyperplasia Due to 17α-hydroxylase Deficiency Since Adolescence: A Case Report

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Abstract

The majority of patients with congenital adrenal hyperplasia (CAH) present with a deficiency of 21-hydroxylase or 11-beta-hydroxylase, which account for 90% and 7% of cases, respectively. However, CAH due to 17α-hydroxylase deficiency (17OHD) is an extremely rare form of CAH (<1% of all CAH cases) that leads to a deficiency of cortisol and sex steroids, along with features of aldosterone excess. This is a case of a 51-year-old single female who was referred to us for the evaluation of new-onset hypertension and hypokalaemia of one-year duration. She was born out of a second-degree consanguineous marriage and reared as a female. She was diagnosed to have testicular feminization syndrome when she presented with a history of primary amenorrhea, absence of secondary sexual characteristics, and bilateral labial swellings at pubertal age. Subsequently, she underwent gonadectomy at the age of 16. Due to the presence of hypertension, metabolic alkalosis and bilaterally enlarged adrenals on CT scan, 46, XY disorders of sexual development (DSD) was considered. A karyotype confirmed the presence of 46, XY chromosomal sex, and genetic analysis revealed a mutation in the *CYP17A1* gene, thus confirming the diagnosis of 17α-hydroxylase deficiency.

Key words: disorders of sexual development (DSD), congenital adrenal hyperplasia, rare cases, hypertension, 46, XY DSD, 17α-hydroxylase deficiency

INTRODUCTION

Disorders of sexual development (DSD) refer to a diverse group of congenital disorders that result in a discrepancy between an individual's sex chromosomes, gonads, and/or the anatomic sex. The 46, XY DSD could be due to a defect in gonadal development or androgen biosynthesis, an error in steroidogenesis as that found in congenital adrenal hyperplasia (CAH), or due to a defect in androgen action.

Congenital adrenal hyperplasia occurs due to enzyme defects involved in adrenal and gonadal steroid biosynthesis. Most CAH cases result from a deficiency of 21-hydroxylase or 11-beta-hydroxylase, accounting for 90% and 7% of cases, respectively.[1,2] Deficiency of 21-hydroxylase and 11-beta-hydroxylase affects only the adrenal steroidogenesis, whereas those with a mutation in 17α-hydroxylase/17,20-lyase (CYP17A1) and 3-β-hydroxysteroid dehydrogenase type 2 (HSD3B2) have an additional impairment in gonadal steroid biosynthesis. Consequently, 21-hydroxylase and 11-beta-hydroxylase cause DSD exclusively in 46, XX individuals. Congenital adrenal hyperplasia due to a mutation in STAR protein, cholesterol side chain cleavage (CYP11A1), and CYP17A1 all cause DSD exclusively in 46, XY individuals. Moreover, deficiencies in HSD3B2 and P450 oxidoreductase cause DSD in both sexes.

Congenital adrenal hyperplasia caused by 17α-hydroxylase deficiency (17OHD) is extremely rare, occurring in approximately 1 in 50,000 individuals.[3] The classical description of 17OHD is that of a phenotypic female (46, XX or underandrogenized 46, XY) who presents at puberty with primary amenorrhea and lack of secondary sexual characteristics, with low-renin hypertension, and hypokalaemic metabolic alkalosis. Here, we report a case of 46, XY DSD due to 17OHD, whose diagnosis was confirmed by *CYP17A1* genotyping. It is important to diagnose this rare condition because persistent hypokalemia in these individuals can lead to life-threatening cardiac arrhythmias, entailing careful monitoring and treatment in the acute phase. In the long-term, there is a risk of osteoporosis if they have not been started on gonadal steroid replacement from the time of puberty. Additionally, they are at a greater risk of developing gonadoblastoma and an invasive mixed germ cell tumor.

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CASE

A 51-year-old single female was referred for evaluation of new-onset hypertension accompanied by hypokalemia with documented bilateral adrenal hyperplasia. She was born out of a second-degree consanguineous marriage and reared as a female. Family history was unremarkable. She had a history of primary amenorrhea, absence of secondary sexual characteristics, and bilateral labial swelling at pubertal age and was mistakenly diagnosed to have testicular feminization syndrome as her karyotype was 46, XY. The patient did not have any gender dysphoria and identified socially as a female. Initially, she was normotensive. The patient and her family members consented to gonadectomy, penile reduction and feminine genitoplasty for this case. Subsequently, when she was 16 years old, she underwent bilateral gonadectomy where her bilateral labioscrotal gonads were removed, and her phallus was surgically reduced.

Given that the patient presented with hypertension, hypokalaemia, metabolic alkalosis, and bilaterally enlarged adrenals on abdominal imaging, the diagnosis of 17OHD was considered. Anthropometry demonstrated eunuchoid body proportion [height 154 cm, weight 51 kg, BMI 21.6 kg/m^2, and upper segment: lower segment ratio 0.94, arm span 175 cm]. Blood pressure was 160/100 mm Hg. Both axillary and pubic hair were absent, and the breast was Tanner stage 1. Genital examination revealed female external genitalia with single urethral opening in the perineum (post-genitoplasty).

Investigations

Serum sodium was 135 mEq/L [normal range (NR) 135-145 mEq/L] and potassium was 2 mEq/L [NR: 3.5-4.5 mEq/L]. Hormonal investigations revealed an 8 am basal cortisol of 3.6 mcg/dl [NR: 4.3-22.4 µg/dL, assay sensitivity (AS) 0.20-75 µg/dL, CV <7%]; plasma adrenocorticotropic hormone (ACTH) 137 pg/ml [NR 0-46 pg/mL, AS: 9 pg/mL, CV<9.6%]; follicle-stimulating hormone 95.24 mIU/mL [NR:1.4-18.1 mIU/mL, AS:0.3 – 200 mIU/mL, CV<4%] and luteinizing hormone 25.74 mIU/mL [NR:1.5-9.3 mIU/mL, AS:0.07 – 200 mIU/mL, CV<3.8%]; serum testosterone <7 ng/dL [NR:14-76 ng/dL, AS:10 – 1500 ng/dL, CV <7.6%]. Bone mineral density was assessed using dual energy X-ray absorptiometry [Hologic Discovery Wi DXA machine] which showed a lumbar spine and femoral neck T score of minus 3.8 and minus 4 respectively.

Computed tomography of the abdomen (Figure 1) showed bilaterally enlarged adrenals and absent Müllerian structures, thus reinforcing the karyotype confirmation of 46,XY DSD. For molecular testing, the patient's consent for a *CYP17A1* gene analysis was obtained and the blood sample sent to the Molecular Endocrinology Laboratory at Christian Medical College, Vellore, India. DNA extraction was carried out on whole blood samples using the Qiagen Gentra Kit method. All the coding exons and splice site regions of the *CYP17A1* gene were amplified using in-house designed primers. Next-generation sequencing with the Ion torrent PGM was performed using previously published protocols.[4] The NGS-based approach achieved an average base coverage depth of 370x, with 99.89% of the target sequenced at 20x, covering the complete coding and splice site regions of the *CYP17A1* gene. The patient was found to be homozygous positive for a reported *CYP17A1*: c.160_162delTTC mutation, resulting in deletion of phenylalanine at codon 54 (p. Phe54del) in exon one, as shown in Figure 2. This mutation has been described in earlier reports from both Japan and New Zealand in patients with similar phenotypes and both 46,XY and 46,XX karyotypes.[5-7] Structural and functional studies of the mutant protein with cos-1 cells lines have shown that this deletion alters the protein folding, thus reducing the activity of 17*α*-hydroxylase (<37%) and 17,20 lyase (<8%) compared to the wildtype protein resulting in

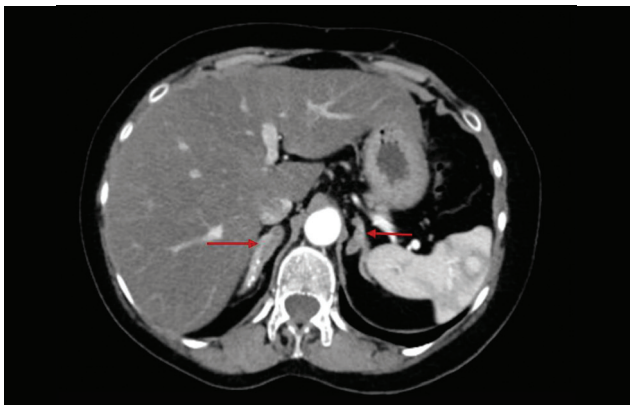

Figure 1. Contrast-enhanced computed tomography of the abdomen showing bilateral adrenal hyperplasia *(red arrows)*.

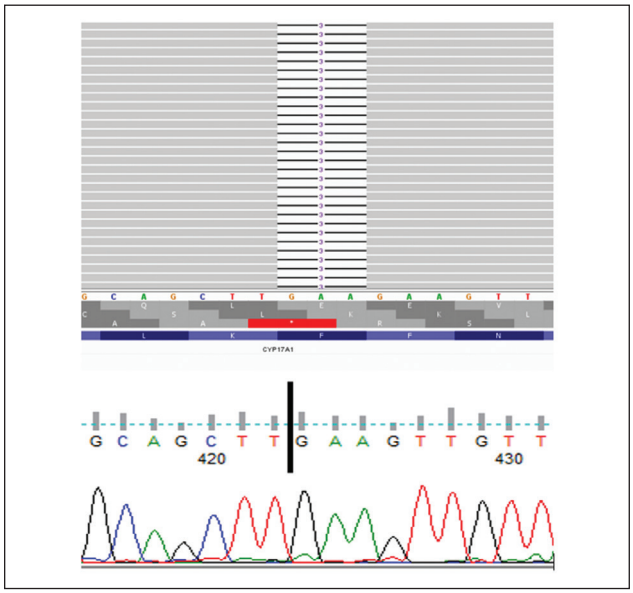

Figure 2. The NGS reads were aligned to hg19 reference genome and viewed on Integrative Genomics Viewer (IGV.2.9.4) as seen in the top image. The gray reads show homozygous deletion of three base pairs (GAA) at codon 54 in exon1 *CYP17A1*(NM_000102.4):c.160_162del (p.Phe54del). The below image shows the sanger chromatogram of *CYP17A1*:P.Phe54del corroborating the NGS results with homozygous deletion of three base pairs (GAA) at codon 54.

partial combined 17α-hydroxylase/17,20 lyase deficiency.[7] Based on the guidelines of the American College of Human Genetics released in 2015, the identified mutation is classified as pathogenic.

Therapeutic intervention and outcome

She was started on a once-daily dose of oral prednisolone (2.5 mg) and spironolactone (50 mg). Additionally, she received a single intravenous infusion of zoledronic acid (4 mg) once yearly dose along with oral calcium (1000 mg daily) for the management of osteoporosis due to long-standing gonadal hormone deficiency. On follow-up, her serum potassium normalized, and her blood pressure was under control.

DISCUSSION

Congenital adrenal hyperplasia due to 17OHD is rare, first described by Biglieri et al.[8] This enzyme is encoded by the *CYP17A1* gene, located on chromosome 10q24.3. Individuals with this form account for about 1% of all CAH cases.[9] A defect in the *CYP17A1* gene results in two different types of enzyme deficiency: (1) combined 17α-hydroxylase/17,20-lyase deficiency and (2) isolated 17,20-lyase deficiency. The combined form of 17OHD is the most common and is characterized by the impaired synthesis of cortisol and gonadal steroids. Depending on the residual enzyme activity (>1% and <1%), it is classified as a combined severe and combined partial form of 17OHD, respectively. Affected 46,XY individuals due to combined severe form of 17OHD classically present with a female phenotype accompanied by primary amenorrhea, absence of secondary sexual characteristics (absent or sparse axillary and pubic hair), female external genitalia with a blind vaginal pouch and variable degrees of hypertension usually detected in adolescence. The gonads (testes) may be located in the intra-abdominal or inguinal region or in the labioscrotal folds. Combined partial form of 17OHD in 46,XY individuals presents with ambiguous genitalia or severe hypospadias with or without hypertension. The normal production of anti-müllerian hormone (AMH) from the testes leads to the regression of müllerian structures (uterus, fallopian tubes and upper one third of the vagina). Affected 46,XX females usually have normal female external and internal genitalia, but the ovaries cannot secrete estrogen during puberty resulting in absent breast development with hypergonadotropic hypogonadism, absent axillary and pubic hair due to lack of adrenal and ovarian androgens. In both 46,XX and 46,XY affected individuals, 17OHD results in impaired synthesis of cortisol and sex steroids with a consequent increase of ACTH and accumulation of steroid precursors, which are diverted into the mineralocorticoid synthesis pathway resulting in the decrease of 17-hydroxypregnenolone, 17-hydroxyprogesterone, 11-deoxycortisol, cortisol, DHEA, androstenedione, estrogen/ testosterone and consequently excess production of DOC, corticosterone, and 18-hydroxycorticosterone respectively. High levels of DOC, due to its mineralocorticoid effect, induce sodium and fluid retention associated with hypertension and hypokalaemia. Despite having a low level of cortisol, patients with 17OHD rarely develop adrenal crisis due to elevated levels of corticosterone, which contributes some glucocorticoid activity, thus preventing an adrenal crisis. Most 17OHD patients have low aldosterone levels caused by elevated levels of DOC, resulting in suppression of the renin-angiotensin system. The isolated 17,20-lyase deficiency is very rare and is characterized by low androgen production leading to genital ambiguity in males, normal production of glucocorticoids and mineralocorticoids.[10]

The differential diagnosis of 46, XY DSD with palpable gonads includes:

1. Defect in gonadal development - partial gonadal dysgenesis.
2. Disorder in androgen synthesis-Leydig cell hypoplasia due to LHCGR mutation, deficiency of STAR, CYP11A1, 3HSDB2, 17OHD, POR, 17βHSD3, 5αreductase type 2.
3. Disorder in androgen action - partial androgen insensitivity syndrome.

A thorough clinical examination is crucial in the evaluation of patients with DSD. As our patient presented with female-type external genitalia, primary amenorrhea, and palpable gonads at puberty along with hypokalemic hypertension and bilateral adrenal hyperplasia, the diagnosis of CAH due to 17α-hydroxylase deficiency (combined severe) was considered since none of the above-mentioned conditions except 17OHD will present with hypokalemia and hypertension in an individual with 46,XY DSD. Laboratory investigations are essential to establish the diagnosis in these settings, which may not be available in many centers; hence, the need to perform these tests in other facilities. We were unable to perform DOC, aldosterone, and renin levels which could reinforce the diagnosis. Nevertheless, as previously described, clinical and available biochemical findings supported the diagnosis of CAH due to 17OHD. The genetic diagnosis in this patient revealed the presence of a deleterious *CYP17A1* mutation. Genetic testing is critical for confirmatory diagnosis of 17α-hydroxylase deficiency due to the presence of varying clinical and biochemical features. Several point mutations, large deletions, and duplications in the *CYP17A1* gene have been reported earlier in the homozygous and compound heterozygous states.[11-14]

The decision about the sex of rearing in DSD is complicated and challenging, especially for those with a 46, XY karyotype. Traditionally, a female phenotype of the external genitalia with palpable gonads results in female sex rearing as it was thought that learning, or "nurture," has a more significant influence on gender identity than biology or "nature."[15] Similarly, our patient was raised and identified as a female as the external genitalia was female with palpable gonads; consequently, feminizing genitoplasty was performed in the past. Another critical issue in the treatment of 46,XY DSD is the appropriate time of gonadectomy. Although data are limited, some reports have shown an increased risk of Sertoli and Leydig cell tumors in phenotypic girls with 17OHD[16] and other forms of 46, XY DSD with undescended testis.[17] Additionally, there have been no reported cases of

biological fertility in individuals with 46,XY DSD due to 17OHD. From a comprehensive perspective, the decision to perform gonadectomy and feminizing genitoplasty was appropriate in this case. However, current guidelines recommend that the decision of gonadectomy should be individualized. Once the sex of the rearing is decided conclusively, the incongruent gonadal tissue can be removed after age 14 years after obtaining informed consent from parents and the concerned individual unless there are compelling indications like suspicion of malignancy.[18]

Most CAH cases, including 17OHD, are inherited in an autosomal recessive form. Hence, there is a 25% probability that other siblings of the index case will have CAH and a 50% probability that each will be an asymptomatic carrier. Genetic testing of other familial members is essential in the recognition of the disease for individualized management as it will aid in sex assignment and estimate the viability of fertility.

CONCLUSION

A 46,XY DSD due to 17OHD may be missed during adolescence. The diagnosis of 17OHD should be considered in a 46,XY DSD presenting with atypical female external genitalia, and absence of secondary sexual characters irrespective of the presence of hypertension, as the presentation of the disorder may vary and hypertension may present later in life. Genetic testing is necessary to confirm the diagnosis of 17OHD. Treatment with glucocorticoids reduces the excess mineralocorticoid precursors and usually normalizes hypokalaemia and hypertension.

Ethical Considerations
Patient consent was obtained before submission of the manuscript.

Statement of Authorship
All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure
The authors declared no conflict of interest.

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CASE REPORT

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Persistent Bilateral Atypical Femoral Fractures in an Antiresorptive-Naïve Singaporean Chinese Patient with Graves' Disease*

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Abstract

Atypical femoral fractures (AFFs) are rare adverse effects of bisphosphonate therapy. We report an unusual case of bilateral diaphyseal AFFs in an antiresorptive-naïve Singaporean Chinese female with Graves' disease. She presented with complete right AFF requiring surgical fixation, and persistent left incomplete AFF for over four years. Femoral bowing, varus femoral geometry, and ethnic influence likely contributed to the AFFs' formation. This case may provide insights into the pathogenesis of AFFs in high-risk Asian populations.

Key words: atypical femoral fracture, bisphosphonate-naïve, antiresorptive-naïve, hyperthyroidism, Asian ethnicity

INTRODUCTION

Atypical femoral fractures (AFFs) are rare stress fractures of the femur with unique radiological and clinical features as outlined by the American Society for Bone and Mineral Research (ASBMR) 2014 Task Force report.[1] They are commonly associated with long-term bisphosphonate (BP) therapy for osteoporosis but are under-recognized in antiresorptive-naïve patients. Although the overall incidence of AFF in the BP-naïve population is low at 0.3 to 0.9 per 100,000 person-years, compared to 55 to 113 per 100,000 person-years in BP users,[1–3] a study on the Southeast Asian (SEA) population revealed that 47.8 % of AFFs were non-BP related.[2]

The risk of AFF is three to six times higher among Asians, with the highest incidence in those from the SEA region.[2,3] Differences in lower limb geometry, genetic variation in bone architecture and BP metabolism have been hypothesized to account for the higher incidence.[3] We report an unusual case of an antiresorptive-naive Singaporean Chinese female with hyperthyroidism who developed bilateral AFFs persisting for four years.

CASE

A 67-year-old Singaporean Chinese female presented in 2019 with a two-day history of right thigh pain after minimal trauma. She fell in a sitting position but could still get up and was able to ambulate with pain. Two days later, she experienced right lower extremity weakness and loss of weight-bearing ability. This was associated with mild, intermittent left thigh pain lasting for one year. She was initially diagnosed with antalgic gait secondary to leg length discrepancy. A bilateral full-length lower limb radiograph was performed in 2018 (Figure 1). Her pain improved and she was lost to follow-up.

Her past medical history included Graves' disease with suboptimal control for 15 years. Her main exercise was aqua aerobics. There was no personal or family history of fracture, autoimmune disease or malignancy. She attained menopause at the age of 50. She was on carbimazole and had never been on bisphosphonates, denosumab, long-term corticosteroids or proton-pump inhibitors.

On examination, her right thigh was tender and swollen. Neurovascular examination of the lower limbs was otherwise unremarkable. She was thyrotoxic but not in thyroid storm. She was overweight by Asian body mass index (BMI) criteria (BMI 24.9, height 164 cm, weight 67 kg). There was no blue sclera, hearing loss, abnormal dentition, or skeletal deformities.

Diagnostic assessment

On admission, a bilateral femoral radiograph was performed (Figure 2) which showed an acute complete right diaphyseal AFF and bilateral chronic incomplete AFFs, in accordance with the ASBMR 2014 criteria.[1] A review

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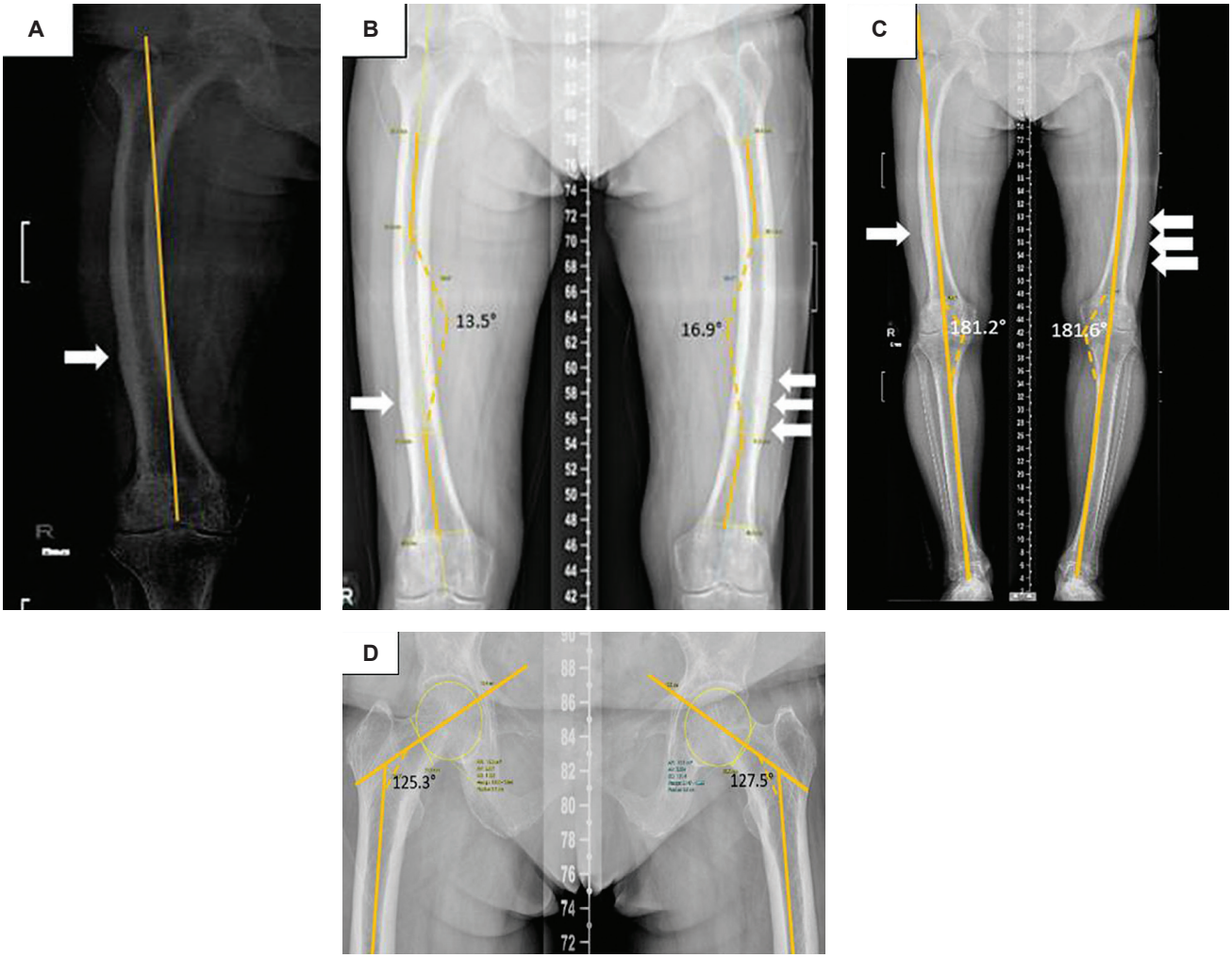

Figure 1. ***Bilateral lower limb full-length radiographs taken in 2018.*** **(A)** Severe (Grade III) lateral femoral bowing, determined by drawing a reference line from the tip of the greater trochanter to the centre of the intercondylar notch and identifying its position being off the medial cortex using Park et al's grading system for anterolateral femoral bowing.[4] **(B)** Lateral femoral bowing angle measured by the angulation between the proximal and distal quarters of the femoral diaphysis using Yau's method[5] showing right and left lateral bowing angle of 13.5° and 16.9° respectively. **(C)** The standing anatomical femorotibial angle (FTA) measured 181.2° on the right and 181.6° on the left *(yellow line)*, suggestive of a varus alignment.[6] **(D)** Femoral neck-shaft angle (FSA) of 125.3° on right and 127.5° on left, consistent with varus hip alignment.[6,7] Multiple transverse linear lucencies *(white arrows)* and cortical thickening at the mid to distal thirds of femoral shafts were seen.

of her old radiographs revealed the presence of bilateral cortical thickening and multiple transverse linear lucent lines at the lateral femoral cortices since 2018, suggestive of chronic stress fractures with bony remodelling (Figure 1).

There was bilateral Grade III (severe) lateral femoral bowing based on the grading system for anterolateral femoral bowing made by Park et al.,[4] with a lateral femoral bowing angle of 13.5° on the right and 16.9° on the left (Figure 1A and 1B).[5] The standing anatomical femorotibial angle (FTA) measured 181.2° on the right and 181.6° on the left, suggesting a varus alignment (Figure 1C).[6] Varus femoral neck-shaft angles (FSA) measuring 125.3° on the right and 127.5° on the left were also seen (Figure 1D).[6,7]

Laboratory investigations (Table 1) showed normal serum parathyroid hormone (PTH), corrected calcium, phosphate, and creatinine levels. She was vitamin D deficient. Thyroid panel showed primary hyperthyroidism. Serum alkaline phosphatase (ALP) and fasting C-telopeptide (CTX) were elevated at 136 U/L (2.27 μkat/L) and 1.11 μg/L respectively. The bone turnover markers may be elevated due to increased bone remodeling during acute AFF healing and may also be due to hyperthyroidism.[1,8] Dual-energy x-ray absorptiometry (DXA) showed osteopenia.

Therapeutic intervention

A Thomas splint was applied while awaiting optimization for surgery. Oral cholecalciferol 50,000 IU once weekly and calcium carbonate 1250 mg BD were initiated. Carbimazole was increased from 15 mg to 40 mg daily, and propranolol 20 mg BD was started for thyrotoxicosis. Following normalization of the FT3 and FT4 levels, she underwent intramedullary nailing (IMN) of the right femur on the fourth hospital day. Surgical fixation of the left femur was

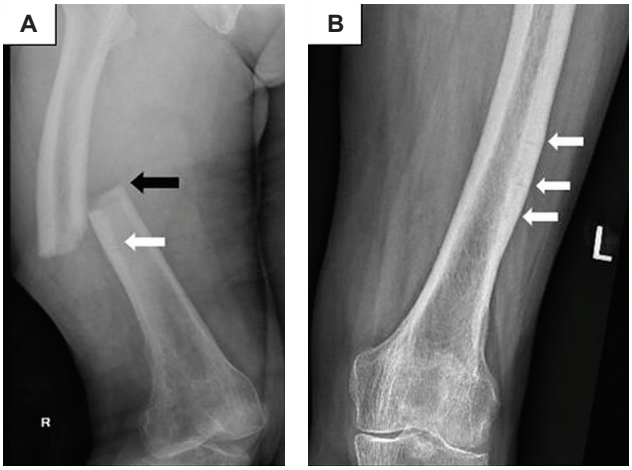

Figure 2. ***Radiographs of bilateral femurs on admission in 2019.*** **(A)** A complete, non-comminuted transverse fracture of the mid right femoral shaft with medial spike *(black arrow)*, consistent with a right complete AFF; and a faint transverse lucent line below indicative of incomplete AFF *(white arrow)*. **(B)** Left incomplete AFFs *(white arrows)* with periosteal cortical thickening and multiple intracortical transverse fracture lines.[1]

not concurrently performed as she was asymptomatic at that time and also with the consideration of the potential difficulties of performing prophylactic nailing on her severely bowed left femur.[4]

Outcome and follow-up

Post-operative recovery was uncomplicated. Carbimazole was tapered once patient achieved euthyroidism, with care to avoid hypothyroidism that may suppress bone turnover and delay AFF healing.[8] Serum 25-hydroxyvitamin D (34.1 ug/L; 85.11 nmol/L), ALP (97 U/L; 1.62 μkat/L), and CTX (0.96 ug/L) levels eventually normalized.

Follow-up imaging showed stable alignment of the IMN and progressive healing of the right AFF (Figure 3A). However, in 2020, she reported a recurrence of left thigh pain and a repeat left femoral radiograph showed persistence of the stress fractures. After a multidisciplinary discussion, she opted for conservative therapy for the left incomplete AFF. She continued to experience intermittent, mild left thigh pain during ambulation. In 2022, 4 years since her first consult, the latest radiographs done showed non-healing lucent lines on the left femur (Figure 3B). Magnetic resonance imaging (MRI) of the left thigh (Figures 3C and D) showed chronic periosteal thickening and sclerosis of the lateral aspects of the distal femoral shaft with areas of serous marrow atrophy, likely secondary to the reparative process from cortical stress fractures. She declined prophylactic nailing of the left femur. Calcium and cholecalciferol supplementation were continued and she was advised to avoid strenuous lower limb activity. She was offered treatment with an anabolic agent (teriparatide) in the hopes of facilitating fracture healing but this was declined due to her concern of the possible adverse effects.

DISCUSSION

The exact pathogenesis of AFFs remains elusive, with several proposed mechanisms. It has been hypothesized that BPs and conditions with low bone turnover predispose to AFF formation by suppressing bone remodeling, which involves (a) osteocyte apoptosis; (b) increased production of receptor activator of nuclear factor-kappa B ligand (RANKL); (c) osteoclastic resorption of damaged tissue and (d) osteoblastic formation to replace resorbed bone.[1] BPs tend to localize at sites with high bone turnover, such as stress fracture sites. They impair targeted remodeling and intracortical repair of these sites, leading to microcrack propagation and fracture.[1] Suppressed bone remodeling may also weaken bone material properties, resulting in harder but more brittle bones with decreased resistance to microcrack progression.[1,7]

Table 1. Laboratory investigations during admission in 2019

Blood investigation	Results	Reference range
Corrected calcium, mmol/L	2.31	2.1- 2.6
Phosphate, mmol/L	1.06	0.65- 1.65
Magnesium, mmol/L	0.83	0.65- 0.95
Creatinine, μmol/L	50	50- 90
Intact Parathyroid hormone (PTH), pmol/L	3.39	1.3- 7.6
25-hydroxyvitamin D, ng/mL	**16.1**	30- 100
Alkaline phosphatase (ALP), U/L	**136**	39- 99
C-telopeptide (CTX), μg/L	**1.11**	Postmenopausal: 0.177-1.015
Free Triiodothyronine (fT3), pmol/L	**6.57**	2.5- 5.5
Free Thyroxine (fT4), pmol/L	**24.33**	10- 20
Thyroid stimulating hormone (TSH), mIU/L	**<0.004**	0.4- 4.0
TSH Receptor Antibody, IU/L	**2.6**	<2.0
Fasting glucose, mmol/L	**6.8**	3.9- 6.0
(mg/dL)	**-122.5**	(70.3- 108.1)
DXA scan:		
• Femoral neck BMD, g/cm^2 (T-score)	**0.696 (-1.1)**	T-score <-1.0 to >-2.5: Osteopenia;
• Total hip BMD, g/cm^2 (T-score)	**0.753 (-1.4)**	T-score <-2.5: Osteoporosis
• Lumbar spine BMD,* g/cm^2 (T-score)	**0.731 (-2.4)**	

* BMD denotes bone mineral density
Abnormal values are indicated in bold. Full blood count, renal panel and liver function tests were unremarkable.

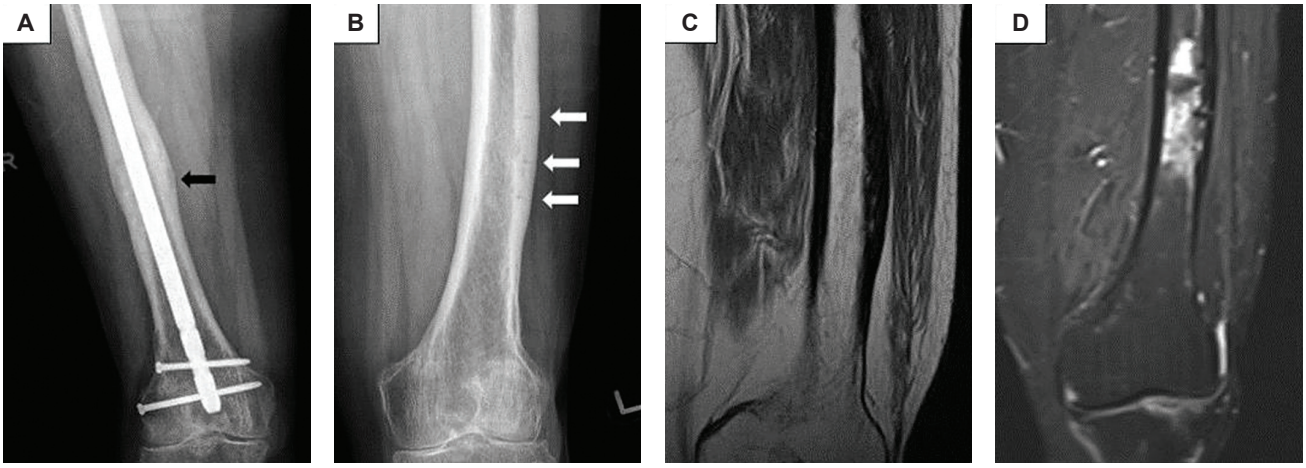

Figure 3. ***Radiographs and Magnetic resonance imaging (MRI) during follow-up visits in 2020 and 2022.*** **(A)** Radiograph of right femur at 10 months (2020) after surgery showing callus formation, progressive healing of right mid-shaft AFF *(black arrow)* and stable alignment of the IMN. **(B)** Radiograph of left femur taken in 2022 showed persistence of the transverse lucent lines and thickened lateral cortex *(white arrows)*, suggestive of persistent incomplete AFFs. **(C)** Turbo spin-echo proton-density weighted (TSE PD) MRI of left thigh showing chronic periosteal thickening and cortical sclerosis of the lateral aspects of the distal femoral shaft while **(D)** Turbo inversion recovery magnitude (TIRM) MRI shows irregular intramedullary high fluid signal of the distal left femoral shaft adjacent to the area of chronic cortical sclerosis, likely reflecting areas of bone marrow oedema secondary to reparative process from atypical cortical stress fractures.

It is interesting that our patient has long-standing hyperthyroidism, a condition usually associated with accelerated bone remodeling.[8] Hyperthyroidism shortens the bone turnover cycle from seven months to three to four months, by increasing the number of osteoclast resorption sites. The ratio of bone resorption to bone formation is increased, leading to cumulative new bone loss and osteoporosis. Unfortunately, we do not have bone histomorphometry analysis of the AFF lesions. To our knowledge, this is the first reported case of persistent AFF in a BP-naïve patient and in a patient with Graves' disease.

Reports of AFFs in patients without prior antiresorptive use or in conditions with low bone turnover suggest alternative mechanisms. The propensity for AFFs to be bilateral and at a similar location on contralateral limbs suggests a relationship between lower limb geometry and AFF formation.[1,7] Patients who developed AFFs had significantly greater anterior and lateral bowing angles of the femoral diaphysis compared to controls and those with typical femoral fractures.[6] Using 2D-3D X-ray scanner EOS™ imaging technology to compare femur geometry, each degree increase in lateral bowing was associated with a 46% increase in AFF risk.[6,7]

The mean femoral anterior radius of curvature in elderly Japanese females was reported as 104 cm compared to 120 cm in Americans. AFF patients were found to have even higher curvatures, with a mean of 59.4 cm (range 48.2 to 81.4 cm).[6] A smaller radius indicates higher femoral curvature. As femur length increases, femoral bowing decreases. This may explain the higher incidence of AFF among Asians, who have shorter femurs and greater bowing compared to Caucasians.

As bowing increases, the area of greatest tensile stress is hypothesized to migrate distally, giving rise to a more distal AFF location.[6,7] The average lateral bowing angle was 10.10° in the diaphyseal group and 3.33° in the subtrochanteric group, with logistic regression analysis showing increased diaphyseal fractures in lateral bowing angles greater than 5.25°.[6]

Although these studies used different methods in measuring femoral bowing, they all showed that anterolateral femoral bowing was associated with an increased risk for diaphyseal AFF. We used the visual grading system by Park et al., (Figure 1A) to identify severe lateral femoral bowing in our patient, as this may be a convenient and practical method for clinicians to identify at-risk femoral curvature in the clinical setting.[4] We also measured the angulation between the proximal and distal quarters of the femoral diaphysis to identify an increase in lateral bowing (Figure 1B).[5]

Other aspects of lower limb geometry hypothesized to influence the risk of AFF are the FSA and FTA. There are suggestions that AFF patients had more varus FSA compared to the non-fractured or typical femoral fracture subjects,[6,7,9] but other studies did not find such an association.[6] Varus FSA of less than 128.3° yielded 69% sensitivity and 63% specificity for the development of AFF.[7] FSA may influence the location of AFF, with smaller FSA in patients with subtrochanteric AFF (125.8°), compared to mid-shaft AFF (130.8°) and non-fractured controls (131.8°).[9] Standing anatomical FTA of patients with diaphyseal AFF were reported to be significantly larger (varus alignment, mean 183.3°) compared to those with subtrochanteric AFF (172.8°), typical femoral fracture and Japanese population cohort (177.6°).[6]

Femoral bowing, varus FSA and FTA alignment in our patient may alter the mechanical axis of the lower limb, leading to concentration of tensile stress in the anterolateral cortex, stress damage, and microcrack formation.[6,7] Over time, this abnormal repetitive loading may go beyond the body's capacity to repair by targeted remodeling, leading to stress fractures.[1] This was supported by biomechanical analysis using quantitative computed tomography/finite element (CT/FE), demonstrating that AFFs occur precisely where maximum tensile stress appears and the location of AFF is determined by individual stress distribution, strongly influenced by femoral bowing and weakly correlated with FSA.[9] Future studies should be done to validate the use of these measurements and grading system to better identify individuals at higher risk of AFF.

Additional factors such as Vitamin D deficiency has been associated with the development of AFF.[1] Serum 25-hydroxyvitamin D concentrations of less than 16 ng/mL increased the risk of subtrochanteric AFF in 1 series (Odds ratio 3.2).[10] Vitamin D deficiency leads to reduced calcium absorption and impaired bone mineralization. The resultant osteomalacia may predispose to femoral bowing and insufficiency fractures.[11] The patient was vitamin D deficient when she presented with right complete AFF, but there was no associated secondary hyperparathyroidism, hypocalcaemia, or hypophosphatemia. Her chest radiograph did not show signs of childhood rickets and she is of normal adult height. Despite repleting her vitamin D and normalization of serum ALP, the left incomplete AFFs persisted for years.

Evidence of genetic influence on AFF was first reported in 3 sisters with AFFs and long-term BP therapy. Whole exome sequencing showed p.Asp188Tyr mutation in the GGPS1 gene in the mevalonate pathway critical to osteoclast function, which is inhibited by BP.[7] Twenty-one rare genetic variants and polygenicity were subsequently reported.[12] AFFs were also found to be associated with 7 monogenetic bone disorders, such as osteogenesis imperfecta, pycnodysostosis, and hypophosphatasia.[12] The patient did not have any clinical features of these conditions; her serum ALP level a few years prior to her AFF was normal. Future studies should look into genetic factors that may play a role in predisposing risk of AFF in the Asian and Singaporean populations. These factors may be associated with bone metabolism and its interaction in the setting of BP exposure.

Previous studies in BP users have reported that only 5% to 18% of non-surgically managed incomplete AFFs showed radiological regression at an average of 11 months to 5.3 years, more frequently when BPs are discontinued.[13] The delayed healing was thought to be due to the long skeletal half-life of BPs and its suppression of intracortical bone remodelling. Our patient was not exposed to BP, but she has lower limb geometries that predispose to increased tensile load on the lateral femoral cortex and she is of high-risk ethnicity, supporting that AFFs are stress fractures that developed from recurrent abnormal loading on the lateral cortex and/or due to inherent factors such as genetic predisposition.

AFFs are associated with increased morbidity, with delayed fracture healing in 26% of cases and a low rate of spontaneous healing among the conservatively managed incomplete AFFs.[1] Symptomatic patients with cortical lucency are at increased risk of progressing to complete fracture and prophylactic nail fixation is recommended.[1,13] However, some patients may opt for non-surgical management, consisting of limitation of weight-bearing activities, adequate calcium and vitamin D replacement, and cessation of antiresorptive medication (where applicable). Anabolic agents, such as teriparatide administration for 1 to 24 months, have been used to accelerate fracture healing in AFFs, although high-quality data on its use is lacking.[1,7] If there is no improvement after 2 to 3 months of conservative management, prophylactic nail fixation should strongly be considered.[1]

CONCLUSION

In summary, we have presented a case of persistent bilateral AFFs in a BP-naïve patient of Singaporean Chinese ethnicity. It highlights the fact that AFFs can be chronic, and the presenting symptoms can be mild and intermittent; subtle early radiographic changes can be easily missed until a precipitant such as low trauma or the initiation of BP therapy leads to a complete fracture. This case may serve to provide a better understanding of the pathogenesis of AFFs, especially the role of mechanical and inherent factors in high-risk ethnic groups. With the rising prevalence of osteoporosis and antiresorptive use, further research is needed to better understand the exact pathogenesis of AFFs and the role of femoral geometry, genetic and clinical risk factors in identifying individuals at high-risk for AFFs. Further studies are also needed in understanding the optimal treatment regimen to prevent future fracture in the high-risk AFF individuals.

Ethical Considerations
Patient consent was obtained before submission of the manuscript.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
KSC: Conceptualization, Validation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **LMD:** Validation, Resources, Data Curation; **LRC:** Validation, Data Curation, Writing – review and editing, Visualization; **LUG:** Conceptualization, Validation, Writing – review and editing, Supervision.

Author Disclosure
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CASE REPORT

Journal of the ASEAN Federation of Endocrine Societies

Sex Reversal Syndrome (SRS): A Case of SRY-Positive 46,XX Testicular Disorder

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Abstract

We report a case of an SRY-positive 46,XX Indian male who presented with small testis and phallus, poor beard and mustache development and gynecomastia at the age of 24 years. He was biochemically found to have hypergonadotropic hypogonadism. He had 46,XX karyotype and Quantitative Fluorescence-PCR (QF-PCR) identified the SRY gene on the X chromosome. SRY-positive 46 XX male SRS cases usually present as phenotypically male since birth but develop features of hypogonadism, poor testicular development, and infertility after puberty. Infertility, hypogonadism, external genital development, and psychological distress are the major concerns during the management of the patients. Testosterone therapy for hypogonadism, artificial reproductive technologies for fertility, surgical repair of hypospadias/cryptorchidism/under-virilized genitalia and psychological and genetic counseling are helpful for proper management of the patients.

Key words: Sex Reversal Syndrome, 46,XX with SRY positive, genetic analysis

INTRODUCTION

Sex Reversal Syndrome (SRS) is a form of gender dysplasia that is characterized by an inconsistency between chromosomal and gonadal sexuality. The clinical types include 46,XY female SRS and 46,XX male SRS.[1] A 46,XX male SRS is a rare clinical condition with a reported incidence of 1 in 20000 newborn males worldwide, but there is no exact data in our country.[2] It most commonly occurs due to the translocation of the Y chromosome including the SRY gene on the X chromosome. Sex-determining region Y (SRY) is the major factor for gonadal differentiation.[3] So, the amount of SRY gene present on the X chromosome and the degree of X chromosome activation determines the genital phenotypic variability. In SRY-negative patients, some other genes in the downstream pathway of testicular differentiation like SOX9, SOX3 and RSPO1 are responsible for gonadal differentiation.[2]

Usually, genitalia development is normal and masculine features are obvious in *SRY+* patients except cryptorchidism, small testis and hypospadias.[4] Though testis morphology is normal in infancy, there is gradual hyalinization with azoospermia and reduced testosterone secretion leading to hypergonadotropic hypogonadism and infertility in adulthood.[5] Clinical phenotypes are somehow similar to Klinefelter's syndrome. However, they are differentiated by their short stature, unlike those with Klinefelter's syndrome who are usually tall and pseudo-eunuchoid.[6] Hypergonadotropic hypogonadism, azoospermia on semen analysis, 46,XX karyotyping and genetic analysis for the presence of the SRY gene help diagnose these patients. Fluorescence in-situ hybridization (FISH) and PCR can be used to identify the SRY gene.

Testosterone replacement therapy is the mainstay of treatment for hypogonadism. Equally important are ensuring psychosexual well-being, prevention of osteoporosis and improving quality of life. In subsequent management, fertility issues and psychological and genetic counseling should be addressed.[7]

CASE

A 24-year-old Indian male consulted an endocrinologist for evaluation of small testis and poor beard/mustache development. His medical history revealed that he had been followed for retractile testis on the left side until the age of 8 years. The patient was born at term after an uneventful pregnancy and there was no parental consanguinity. He had a normal libido, good erectile function with normal morning erections and no genital or urinary troubles. He complained of mild asthenia, impaired concentration and breast development in the last 2 years. There was no family history of infertility or other genetic disorders. His Intelligence Quotient (IQ) level was normal.

The physical examination revealed a eunuchoid body habitus (height: 171 cm; weight: 64 kg; arm span: 179 cm; US:LS: 0.85 and a normal male appearance but with scanty

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body, axillary and facial hair. The patient had normal male pubic hair distribution, gynecomastia and normal male external genitalia with no hypospadias and cryptorchidism. Pubic hair Tanner stage was 5 (P5) with a stretched penile length of 10 cm, testicular volume of 6 ml and soft atrophic testis bilaterally. Testicular ultrasonography showed bilateral small testis; the bipolar length of both the right and left testes is about 12 mm. Pelvic ultrasonography showed normal male internal genitalia without any Mullerian derivatives. There was bilateral mild varicocele (Grade l). On semen analysis, the semen sample was translucent and contained no sperm (azoospermia). Hormonal investigations revealed hypergonadotropic hypogonadism (Table 1).

Due to hypergonadotropic hypogonadism, karyotype analysis was performed in two different laboratories revealing 46,XX (Figure 1). For karyotyping, 50 blood lymphocytes were examined at the metaphase stage, where the probability of mosaicism detection was high (around 100%). Subsequently, quantitative fluorescence polymerase chain reaction (QF-PCR) identified the SRY gene on the X chromosome (Figure 2). QF-PCR analysis includes amplification, detection, and analysis of short tandem repeat (STR) markers and non-polymorphic markers. Fluorescently labeled primers are used for the amplification of chromosome-specific markers and, thus, each marker's copy number indicates the copy number of the chromosome. When a chromosome-specific STR marker is heterozygous, two peaks in a 1:1 ratio are found, and when the marker is homozygous, only one peak is observed. The existence of an extra allele as three peaks in a 1:1:1 ratio or two peaks in a 2:1 or 1:2 ratio suggest the presence of an additional STR sequence, which may relate to a different chromosome.[8] A testicular biopsy was proposed to get a histological diagnosis, but the patient refused.

Table 1. Patient's hormone profile

Laboratory Test	Results	Normal values
LH (mIU/ml)	21.60	Adult male: 2.00 - 12.00
FSH (mIU/ml)	22.00	Adult male: 1.00 - 08.00
S. Testosterone (nmol/L)	2.82	20-50 years old (male): 10.40 - 35.71
S. Estradiol (pg/mL)	20.08	Male: 0.00 - 84.00

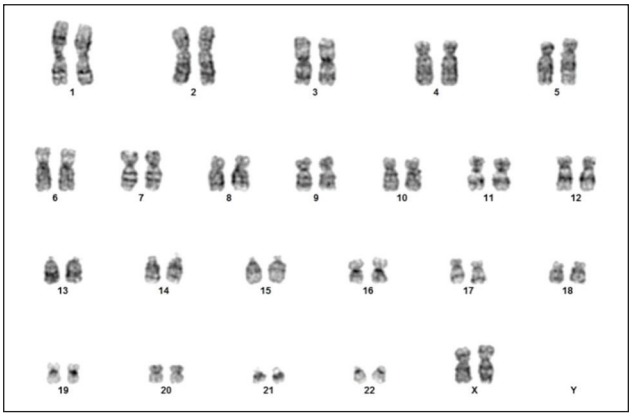

Figure 1. Result of karyotype analysis revealing 46,XX.

Testosterone replacement therapy, in a dose of 250 mg per month, was initiated along with genetic counseling. He was referred to a psychiatrist for psychological counseling.

DISCUSSION

Human sexual development is a complex process determined by different chromosomal and genetic factors and their degree of expression. In the first stage of sexual development, gonadal differentiation is largely determined by the presence of the SRY gene and the subsequent differentiation into genitals. The formation of secondary sex characteristics is dependent on the hormone secreted by the differentiated gonad.[1] Three mechanisms have been postulated for the etiology of 46,XX male DSD: (1) Translocation of Y chromosome including the SRY gene on the X chromosome or on autosomal chromosomes; (2) Secret Y mosaicism found only in the gonads; and (3) X-linked mutation/ overexpression in the genes that cause testis differentiation or mutation/ overexpression in autosomal genes [e.g., SRY box-related gene 9 (SOX9)] in SRY negative XX males.[3] The most common type, translocation of SRY locus containing the Y chromosome on the X chromosome, occurs during paternal meiosis recombination.[2] Due to phenotypic difference, 46,XX males can be clinically divided into the SRY-positive and the SRY-negative groups. SRY-positive 46,XX patients usually present in adulthood with normal-appearing male external genitalia and masculine signs but with small testes and phallus. Genital ambiguity can also be seen in the former, whereas SRY-negative patients usually present with genital ambiguity since birth and varying degrees of masculinization.[2,9] Phenotypes in these patients depend largely, but not only, on the presence of the *SRY*. Other genes like *SOX9*, *SOX3*, *DAX1*, *WT1*, *FGF9*, and *SF1* are also involved in the downregulatory pathway of the sex determination cascade.[6]

Phenotypic variability of SRY-positive 46,XX subjects depends on the amount of Y materials, its position on the X chromosome or the presence of minor deletions or point mutations secondary to the exchange of genetic material and activation of the translocated X chromosome for sufficient expression of the SRY protein.[10] Usually, genitalia development is normal and masculinity signs are obvious in *SRY*+ patients. In the index case genital development, signs of masculinization, erection, ejaculation and sex psychology were also normal but there was a small penis, retractile testis before puberty, gynecomastia, poor beard and mustache development. So, based on phenotypic abnormality, it is difficult to find *SRY*+ male DSD patients before puberty.[4] Like the other 46,XX testicular DSD patients, testis morphology was normal in infancy and there was little increase in testicular size to 6 mL in adulthood. Hyalinization of the seminiferous tubules in early childhood causes loss of spermatogonia. So, the level of testosterone is normal during puberty, but deficiency gradually develops during adulthood leading to hypergonadotropic hypogonadism and infertility.[5] Though SRY-positive 46,XX patients present with hypergonadotropic hypogonadism

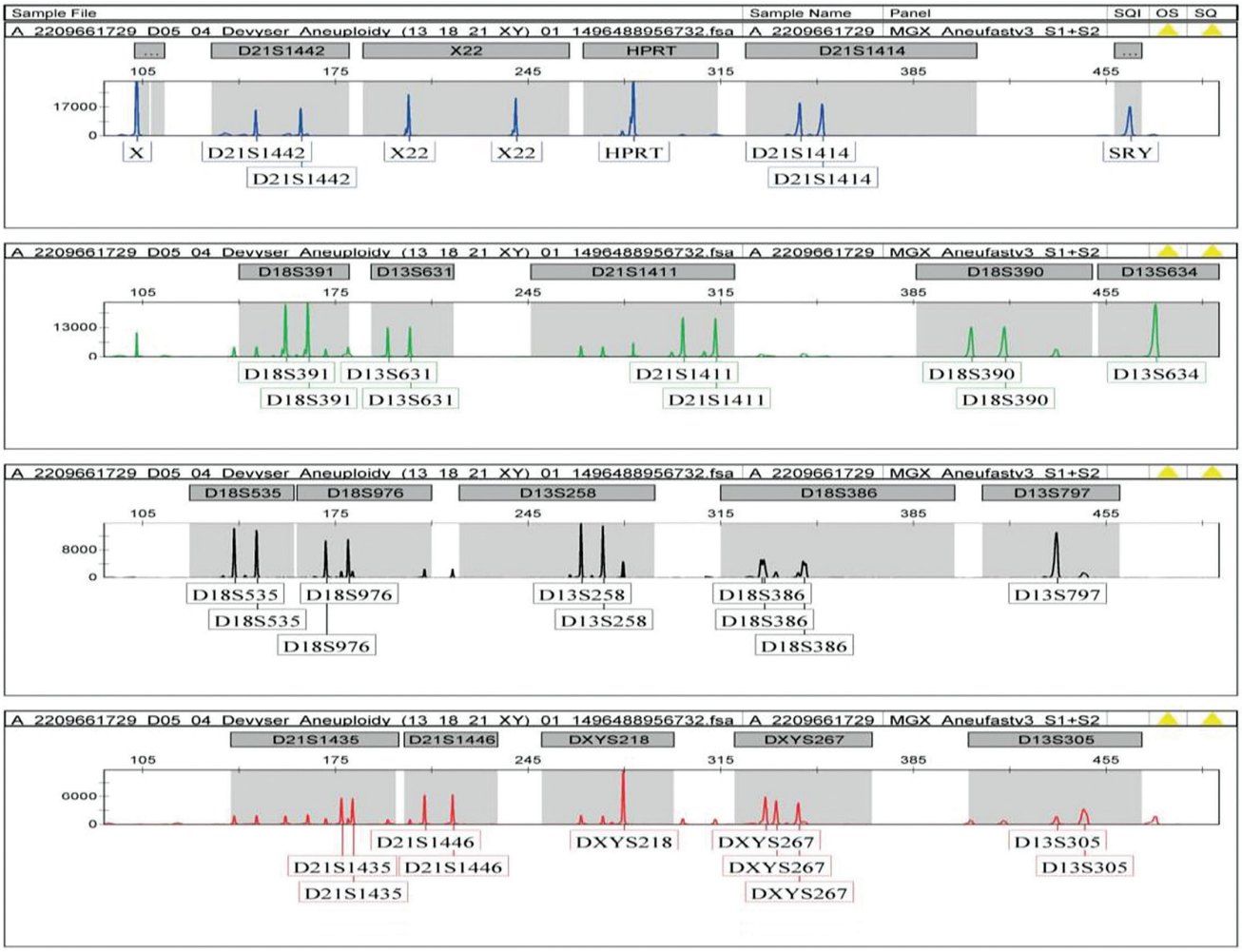

Figure 2. Quantitative fluorescence-PCR (QF-PCR): Upper circle- SRY gene, Lower circle - copies of both X and part of the Y chromosome.

and small testes similar to Klinefelter's syndrome (47,XXY), they are differentiated by short stature, unlike those with Klinefelter's syndrome who are usually tall and pseudo-eunuchoid.[6] In the reported case, there was eunuchoid body habitus which may occur if the hypogonadism develops before puberty.

For diagnosis, appropriate physical examination, trans-abdominal ultrasonography and karyotypic analysis with detection of the SRY gene are required. Semen analysis is mandatory which usually shows azoospermia and so is the karyotype test.[6] FISH and PCR technology can quickly and accurately detect the information about the SRY gene in patients.[1] We identified the SRY gene by QF-PCR method and diagnosed the case as 46,XX male. Phenotypic variability of SRY-positive 46,XX patients, may be explained by the differential inactivation of the X chromosome carrying SRY, thereby influencing SRY expression. X-chromosome inactivation patterns (XCIP) can be assessed by X replication studies, in situ hybridization associated with a modified R-banding technique, or methylation-sensitive PCR analysis of sequences of the androgen receptors (AR) gene, on the active and inactive X chromosomes.[9] So, these techniques help to explain the genotype-phenotype relationship in this group of disorders. These are not available in our country, so the X-chromosome inactivation pattern was not done. During the evaluation of a phenotypically male subject with small firm testis, small phallic length and biochemically hypergonadotropic hypogonadism, karyotyping with detection of the SRY gene is necessary to establish the diagnosis and for the exclusion of other differentials.

Management is based on the presenting signs and symptoms, fertility issues, psychological and genetic counseling and prevention of long-term complications due to hypogonadism. The mainstay of treatment is testosterone replacement therapy to correct hormonal imbalance, prevent gynecomastia and osteopenia/ osteoporosis and maintain normal sexual life as well as improve quality of life. Reduction mammoplasty may be considered in some cases.[7] Psychological support and timely referral to an assisted conception service should be offered. Management options for infertility in couples where the male has 46,XX testicular DSD is artificial reproductive technology including artificial insemination of the female partner with donor sperm. Growth hormone therapy may be considered for short stature.

Surgical repair of orchidopexy and/ or hypospadias, if under-virilized, should be offered. As the patients are phenotypically and psychosexually male, their main psychological distress stems from infertility, sexual dysfunction due to hypogonadism, genotype-phenotype aberration and external genital development (poorly developed to ambiguous).[7] Referral to a mental health professional is, therefore, imperative. Utmost sensitivity is necessary when conveying information about the genetic cause of the disorder and associated sterility. SRY-positive 46,XX testicular DSD is generally not inherited because of infertility of patients and the *de novo* occurrence of Y and X chromosome translocation. The SRY gene, in rare occasions, may be positioned incorrectly on a chromosome other than the X chromosome. This translocation may be carried by an unaffected father and passed on to a child with two X chromosomes, resulting in 46,XX testicular difference of sex development.[10] In SRY-negative cases, the pattern of inheritance depends on the genetic cause, if known. So, during genetic counseling, the mode of inheritance should be addressed.

CONCLUSION

SRY-positive 46,XX males phenotypically present with normal male external genitalia and signs of masculinization except cryptorchidism, small testes, small phallus, and hypospadias. They usually present in adults with small testis, small phallus, hypergonadotropic hypogonadism and azoospermic infertility, similar to Klinefelter's syndrome. For this reason, a phenotypic male with small firm testes, small phallus and hypergonadotropic hypogonadism should have a karyotype done to distinguish from Klinefelter's syndrome (47,XXY) or KS mosaics. If karyotype 46,XX is determined, testing for the presence of the SRY gene is necessary to establish the diagnosis and subsequent management. Infertility, hypogonadism, external genital development, and psychological distress are the major concerns during the management of the patients. Testosterone therapy for hypogonadism, artificial reproductive technologies for fertility, surgical repair of hypospadias/cryptorchidism/under-virilized genitalia and psychological and genetic counseling are helpful for proper management of the patients.

Ethical Considerations
Patient consent was obtained before the submission of the manuscript.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
KS: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **TF:** Conceptualization, Validation, Writing – review and editing; **TH:** Conceptualization, Validation, Writing – original draft preparation, Writing – review and editing; **MH:** Conceptualization, Methodology, Validation, Investigation, Writing – review and editing, Supervision, Project administration.

Conflict of Interest
The authors have no conflicts of interest to disclose.

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CASE REPORT

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Persistent Primary Hyperparathyroidism Secondary to an Ectopic Mediastinal Adenoma in a Young Adult: A Case Report

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Abstract

Primary hyperparathyroidism commonly affects elderly women. When present in the young population, it is usually asymptomatic, most frequently due to a parathyroid adenoma and the definitive management is surgical excision. Uncommonly, 5-10% of patients fail to achieve long-term cure after initial parathyroidectomy and 6-16% of them is due to an ectopic parathyroid adenoma that will require focused diagnostic and surgical approaches.

We report a 21-year-old male who had bilateral thigh pain. Work-up revealed bilateral femoral fractures, brown tumors on the arms and multiple lytic lesions on the skull. Serum studies showed hypercalcemia (1.83 mmol/L), elevated parathyroid hormone [(PTH) 2025.10 pg/mL], elevated alkaline phosphatase (830 U/L), normal phosphorus (0.92 mmol/L) and low vitamin D levels (18.50 ng/mL). Bone densitometry showed osteoporotic findings. Sestamibi scan showed uptake on the left superior mediastinal region consistent with an ectopic parathyroid adenoma. Vitamin D supplementation was started pre-operatively. Patient underwent parathyroidectomy with neck exploration; however, the pathologic adenoma was not visualized and PTH levels remained elevated post-operatively. Chest computed tomography with intravenous contrast was performed revealing a mediastinal location of the adenoma. A repeat parathyroidectomy was done, with successful identification of the adenoma resulting in a significant drop in PTH and calcium levels. Patient experienced hungry bone syndrome post-operatively and was managed with calcium and magnesium supplementation. A high index of suspicion for an ectopic adenoma is warranted for patients presenting with hypercalcemia and secondary osteoporosis if there is persistent PTH elevation after initial surgical intervention. Adequate follow-up and monitoring is also needed starting immediately in the post-operative period to manage possible complications such as hungry bone syndrome.

Key words: hyperparathyroidism, reoperation, hypercalcemia, ectopic parathyroid adenoma

INTRODUCTION

Primary hyperparathyroidism (PHPT) is a common disorder of mineral and bone metabolism that often presents with high calcium levels with elevated parathyroid hormone (PTH).[1] This may result in kidney stones and pathologic fractures. Once treated, 5-10% of patients may fail to achieve long-term cure after initial parathyroidectomy[2] and is considered persistent hyperparathyroidism if calcium and PTH are still elevated within 6 months of neck exploration. One of the reasons includes an ectopic location of the gland[3] – which only occurs in 6-16% of patients[4] such as in our case.

CASE

This is a 21-year-old Filipino male, with no known comorbidities presenting at the emergency room due to severe pain of both thighs. Three months prior, the patient experienced bilateral leg and thigh pain with no prior trauma or injury. No consult was done until 1 day prior to consult while he was walking on the pavement, he suddenly felt his legs give way, causing him to fall and land on his buttocks. The patient had no previous admissions and had an unremarkable past medical, personal-social, genetic and family history. He had a BMI of 17.3 kg/m^2 and physical examination showed no remarkable findings other than pain on movement.

On work-up, skeletal survey revealed brown tumors on the arms (Figure 1) and multiple nonaggressive lytic lesions on the skull. His legs had multiple fractures described as complete linear displaced fracture at the left femoral neck and complete oblique fracture at the left and right femoral proximal shafts (Figure 2). Laboratory tests showed elevated calcium with a normal phosphorus and low 25-hydroxyvitamin D level. Additional work-up was then performed with a consideration of PHPT. Bioactive parathyroid hormone (PTH) assay was requested twice, showing markedly elevated levels. Other remarkable findings were a low magnesium, elevated alkaline phosphatase (ALP), elevated urinary calcium excretion and presence of non-obstructing nephrolithiasis on the left (Table 1). Bone densitometry also showed z-scores

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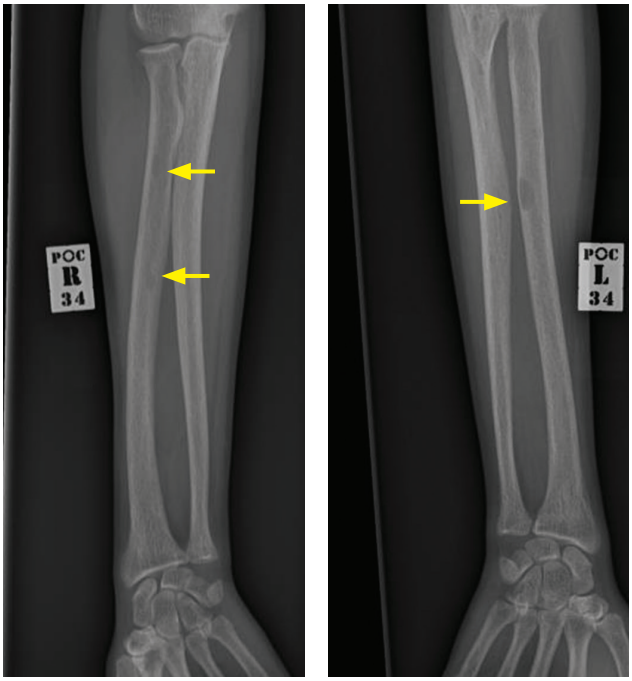

Figure 1. Technetium 99m sestamibi scan shows intense focal tracer uptake and retention in the left superior mediastinal region.

below the expected range for age. With a high suspicion of PHPT, a technetium 99m sestamibi scan was performed which revealed an intense focal sestamibi accumulation and retention in the left superior mediastinal region consistent with an ectopic parathyroid adenoma (Figure 3). This prompted transfer to our institution for surgical intervention.

The patient was referred to a head and neck surgeon and underwent parathyroidectomy and neck exploration with frozen section via low collar incision. Fifteen minutes after excision of the suspicious parathyroid adenoma, PTH assay was done showing persistently elevated levels. Frozen section identified thymic tissue and benign thyroidal tissue. Neck exploration to remove other suspicious lesions was then performed. PTH level 15 minutes thereafter remained elevated and frozen section only identified unremarkable lymph nodes. The procedure was terminated with plan to reopen once further imaging is performed.

To further localize the parathyroid adenoma, a computed tomography (CT) scan with intravenous contrast was performed on the neck and chest (Figure 4) which showed an avidly enhancing soft tissue nodule in the aorto-pulmonary window/anterior left mediastinum region

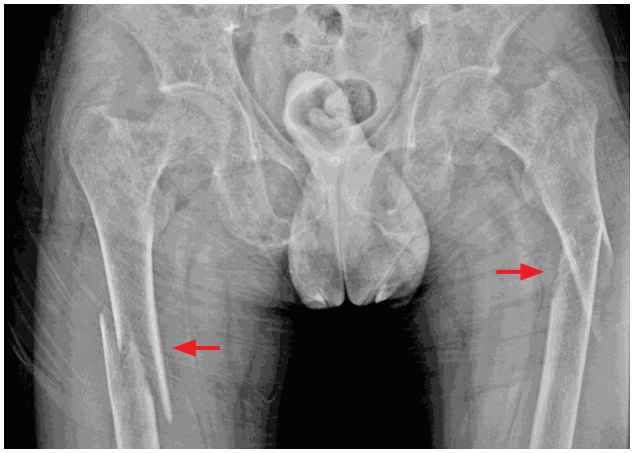

Figure 2. Frontal radiograph of both upper femurs shows badly displaced fractures of both upper femoral shafts *(red arrows)*. These pathological fractures have occurred through bilateral underlying osteolytic lesions consistent with brown tumors.

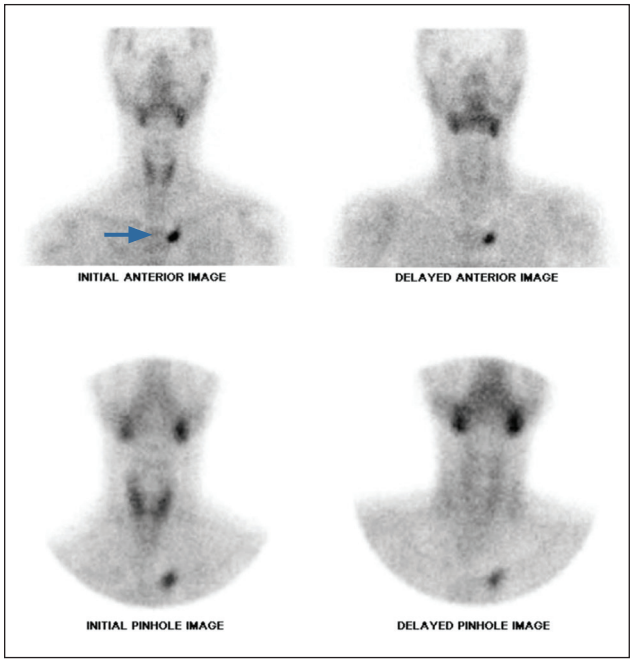

Figure 3. Technetium 99m sestamibi scan shows intense focal tracer uptake and retention in the left superior mediastinal region.

consistent with a thymoma and/or residual ectopic parathyroid adenoma. The second operation was then performed with a referral to a thoracic and cardio-vascular surgeon in order to proceed with an ectopic parathyroidectomy via midline sternal incision. To aid with

Table 1. Pertinent laboratory findings

	First operative attempt			After second operative attempt		
	Day 1	Post parathyroidectomy with frozen section	After neck exploration	Day 1	Day 2	Day 3
Magnesium (Normal Value: 1.8-2.6 mg/dL)	1.41	1.68		1.43	1.39	1.29
Ionized Calcium (Normal Value: 1.12-1.32 (mmol/L)	1.83	1.60		1.23	0.93	1.07
PTH (Normal Value: 15-65 pg/mL)	2025.10	1553.90	2180.40	208.80		

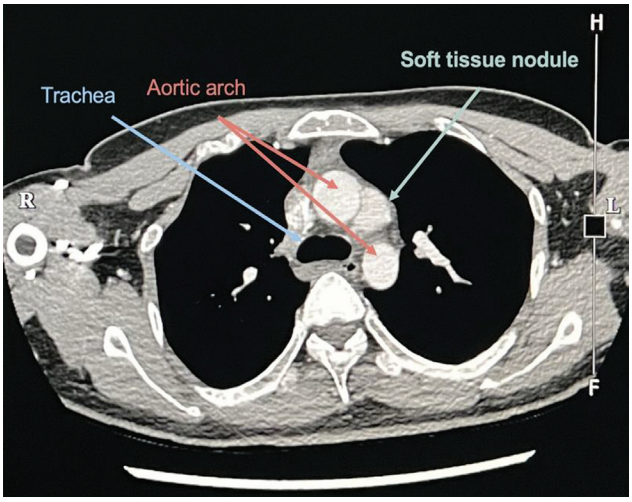

Figure 4. Axial contrast-enhanced CT image shows an enhancing soft tissue nodule in the aortopulmonary window *(labelled)* consistent with an ectopic parathyroid adenoma. This corresponds to the focus of tracer uptake detected on the Tc-99m sestamibi scan.

localization, technetium 99 m sestamibi was administered prior to the procedure and a gamma probe was utilized to confirm the presence of the adenoma. Fifteen minutes after excision of the suspected adenoma, PTH markedly dropped by 89%. Frozen section further confirmed parathyroid adenoma. On gross examination, the specimen obtained was a light brown to light red, irregular, soft to rubbery tissue measuring 2.2 x 1.8 x 0.7 cm and weighing 2.0 grams. Final histopathology report revealed a parathyroid adenoma.

Immediately postoperatively, the patient was started on calcium gluconate drip and transitioned to calcium tablet once he was able to tolerate oral medications together with calcitriol and magnesium supplementation. Ionized calcium and magnesium levels were monitored postoperatively and medications were titrated accordingly. Patient was then cleared for discharge without signs and symptoms of hypocalcemia. On follow-up after 1 week, calcium levels were still low hence, calcitriol was adjusted and compliance to the regimen provided was emphasized. Fracture repair is still on hold while waiting for the prostheses from social services but is planned to be done immediately once available.

DISCUSSION

PHPT is predominant among postmenopausal women although the prevalence varies by country and race.[5] In countries such as the Philippines, screening is not routine hence, PHPT is infrequently seen. According to a report by Lou et al.,[6] the incidence of PHPT is less common in the young (2-5 cases per 100,000) and were usually asymptomatic, unlike in our case.

Hypercalcemia is usually caused by either PHPT or malignancy in 90% of patients.[7] Hence, the approach to diagnosis is to distinguish between the two by using serum PTH. A high serum PTH in the background of hypercalcemia is likely due to PHPT[8] and is most commonly due to an adenoma. Ectopic adenomas have been reported to account for only 4 to 16 % of patients with hyperparathyroidism and are classically described as occurring anywhere from the angle of the mandible to the mediastinum.[9]

Since our patient had elevated serum calcium on top of elevated PTH levels, ultrasound of the neck and sestamibi scan was done to locate the causative etiology similar to the recommendation of Roy et al., for localization in unexplored patients (REF).[4] This then revealed an adenoma in the left superior mediastinal area which the head and neck surgeon operated on with a consideration of an intrathymic parathyroid adenoma based on the imaging studies done.

Due to the persistently elevated PTH levels and histopathology report of thyroid and thymic tissues, a CT scan of the neck and chest was done. This is in line with the algorithm proposed by Yen et al.,[10] for further localization before a second operation. It was recommended that if there is persistent or recurrent PHPT localized with sestamibi studies, a CT or MRI may be done and once with localization, re-operation may be performed. If additional imaging yields no findings, parathyroid angiography with selective vein sampling may be considered. If the lesion remains elusive, conservative management is advised. This was applied to our case, where a CT was done prior to re-operation and revealed a focus on the superior mediastinal area consistent with a residual ectopic parathyroid adenoma.

Surgery is the definitive management of adenoma(s) and when presented with an ectopic location, imaging studies can be used to further locate the lesion. However, the ultimate factor in the success of operations would still be surgical volume as shown by Zarebczan et al.[11] They reported cure rates as high as 95% in initial operations but decreases to 80% for re-operation cases. However, the use of SPECT-CT may be able to localize the ectopic lesions more precisely.[12] The limiting factor for its use in this case was the availability and cost of the SPECT-CT. On a side note, intraoperative gamma probe, PTH monitoring and frozen section were facilitated by the team in this case which contributed to the success of the operation. The PTH levels normalized post-operatively which is consistent with the studies performed for these various intraoperative adjuncts.[13-15]

Post-operatively, hungry bone syndrome (HBS) was observed in this case and was anticipated due to the presence of risk factors such as elevated ALP and high pre-operative PTH levels consistent with the study of Bollerslev et al.[16] Calcium replacement may be done intravenously for symptomatic patients or if there are ECG changes consistent with hypocalcemia. Oral calcium supplements at a range of 6-12 g/day may be given for asymptomatic patients.[17] Magnesium should be replaced if needed since persistently low magnesium would hinder calcium replacement because it may impair PTH production leading to a state resembling hypoparathyroidism.[18]

As to its prognosis, there appears to be a great degree of variability regarding how long it can last. In some case reports, the need for calcium and active vitamin D replacement may last for up to 1 year post-operatively.[19] Hence, follow-up care should also be emphasized to adjust the medications prescribed, with proper monitoring of calcium level post-operatively to avoid further complications.

CONCLUSION

Since calcium levels are frequently included in general laboratory screens, hyperparathyroidism predominantly presents asymptomatically and is common in elderly females. However, in low to middle-income countries, classic manifestations of hyperparathyroidism may be seen, even in the young. Hence, a high index of suspicion is warranted for patients presenting with hypercalcemia and secondary osteoporosis. Furthermore, an ectopic adenoma should be considered especially with persistent PTH elevation after initial surgical intervention. Complete laboratory workups and imaging studies should be performed to better localize the pathologic adenoma. We highlight the value of additional diagnostic tests including CT scan of the neck and chest, intraoperative gamma probe, serial PTH monitoring and frozen section in the success of surgery.

Ethical Considerations
Patient consent was obtained before submission of the manuscript.

Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
KHN: Conceptualization, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision; **RA:** Conceptualization, Validation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision.

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CASE REPORT

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Langerhans Cell Histiocytosis Presenting as Anterior Neck Mass in a Child: A Case Report*

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Abstract

Thyroid involvement in Langerhans Cell Histiocytosis (LCH) is rare. We report a 10-year-old Filipino male who presented with a rapidly enlarging goiter. Computed tomography scan showed thyroid and bilateral submandibular masses with malignant features, pulmonary blebs and hepatic cysts. Ultrasound-guided core needle biopsy findings were consistent with LCH and chemotherapy was initiated. This case demonstrates that LCH should be considered in patients with goiter. Multidisciplinary management is warranted to achieve proper diagnosis and institute timely treatment.

Key words: Langerhans Cell Histiocytosis, thyroid, multisystem LCH

INTRODUCTION

Langerhans cell histiocytosis (LCH) is an inflammatory myeloid neoplasia characterized by accumulation of pathologic Langerhans cells into organs.[1] These langerin-positive (CD207+) histiocytes may develop in any tissue, but a study of LCH organ involvement showed that the incidence is highest at the skeletal system at 80%, followed by the skin (60%), and lymph nodes (33%).[2] In children, LCH is usually a multisystemic disease, and involvement of the thyroid gland is extremely rare.[3]

CASE

A 10-year-old Filipino male consulted at the outpatient Pediatric Endocrinology clinic with a two-month history of a rapidly enlarging anterior neck mass. There was no dysphagia, hoarseness, cold or heat intolerance, or bowel movement changes. He had a height of 133 cm which was appropriate for age. Physical examination showed an anterior neck mass measuring 15 x 16 x 4 cm (L x H x W). Thyroid ultrasound showed thyroid gland with coarsened and nodular echopattern and enlargement of both submandibular glands. Thyroid function tests were normal with a FT3 of 2.45 pg/ml (reference range: 2-4.35), FT4 of 14.77 pg/ml (reference range: 7-18) and TSH of 3.63 uIU/ml (reference range: 0.25-4). After two weeks, the Otorhinolaryngology service performed fine needle aspiration biopsy. The fine needle aspiration cytology (FNAC) result was Bethesda system category IV: suspicious for follicular carcinoma and advised correlation with clinical and other radiographical findings. After one month, during which the patient had weight loss, poor appetite and dysphagia, he was admitted for possible thyroidectomy with neck dissection.

Family history revealed goiter on the maternal side. Past medical history revealed that three months prior to anterior neck enlargement, he had a history of recurrent bilateral spontaneous pneumothorax requiring video-assisted thoracoscopic surgery (VATS), blebectomy, and mechanical and chemical pleurodesis.

Hematology-oncology service and a pediatric airway team consisting of Critical Care, Pulmonology, Otorhinolaryngology, Anesthesiology, and Surgery co-managed the patient. Complete blood count showed anemia, leukocytosis with neutrophilic predominance, and thrombocytosis. Electrolytes and arterial blood gas were normal. Chest radiograph (Figure 1) revealed right pneumothorax. Urinalysis showed microscopic hematuria and pyuria. Repeat thyroid function tests showed normal FT3 of 4.2 pmol/L (reference range: 3.1-6.8), normal FT4 of 17.3 pmol/L (reference range: 12-22) and elevated TSH of 19.6 uIU/mL (reference range: 0.27-4.2). Computed tomography scan of the neck and chest (Figure 2) revealed thyroid mass

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with malignant features and local mass effect, invading the right internal jugular vein and superior vena cava. In addition, there were pulmonary blebs and multiple hepatic cysts that are not typical features of thyroid carcinoma.

Interventional radiology performed an ultrasound-guided core needle biopsy of the thyroid and lymph nodes, which showed atypical, medium-sized cells with abundant amphophilic to eosinophilic cytoplasm, centrally located, and grooved nuclei. These atypical cells are admixed with abundant eosinophils and showed strong immunoreactivity to CD1a, S100, and Langerin (Figure 3), which strongly supports a diagnosis of LCH. The previous cell block of his excised bleb was also reviewed and showed CD1a positivity, compatible with LCH. Bone marrow aspiration with biopsy and cranial CT scan showed normal results.

During admission, the patient initially received an anti-inflammatory dose of prednisone at 1 mg/kg/day, antibiotics for urinary tract infection, and levothyroxine at 2.1 mcg/kg/day for subclinical hypothyroidism. The patient developed pneumothorax prompting endotracheal intubation and chest tube thoracostomy. Upon stabilization, chemotherapy was initiated with the following treatment protocol: vinblastine 6 mg/m^2 once a week for 6 weeks and prednisone 40 mg/m^2 divided into 3 doses for 4 weeks. There was a significant decrease in goiter size within two weeks of chemotherapy. Three months after initiation of chemotherapy, thyroid function test showed normal FT3 of 4.89 pmol/L (reference range: 3.1-6.8), elevated FT4 of 22.35 pmol/L (reference range: 12-22) and TSH of 2.14 uIU/mL (reference range: 0.27-4.2). Levothyroxine was decreased to 1.7 mcg/kg/day. It was discontinued after a month in preparation for thyroid scan, which revealed normal sized, normofunctioning thyroid gland. Seven weeks after levothyroxine discontinuation, thyroid function test showed normal FT4 of 19.38 pmol/L (reference range: 12-22) and elevated TSH of 7.98 uIU/mL (reference range: 0.27-4.1). He denied cold intolerance, constipation, nor easy fatigability. Thus, the service opted to observe subclinical hypothyroidism.

The patient was noted to have polydipsia and polyuria with negative fluid balance. Central diabetes insipidus (DI) was considered, however, results of the water deprivation test was inconclusive with serum osmolality of 287.7 mOsm/kg and urine osmolality of 560 mOsm/kg. The test had to be terminated when the patient lost 5% of his weight. Hence, further monitoring was suggested. Cranial MRI was requested showing a normal pituitary bright spot (Figure 4). The patient was discharged and is currently on continuation phase of his chemotherapy.

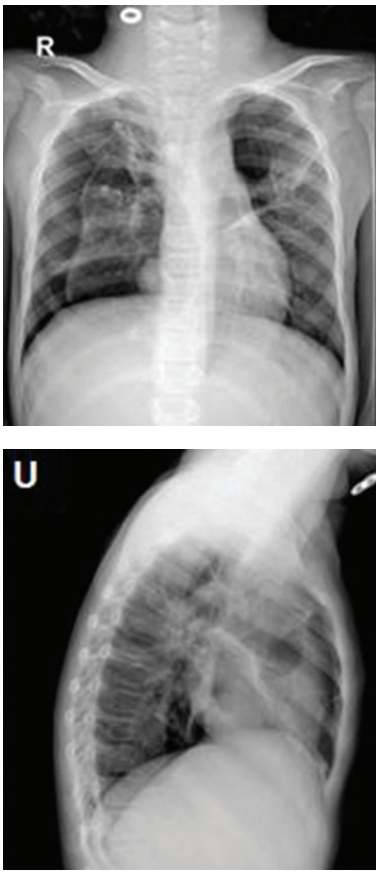

Figure 1. Chest radiograph showed pneumothorax on the right hemithorax, ovoid density on left upper lobe possibly bullous changes, and possible segmental atelectasis on the left hilum.

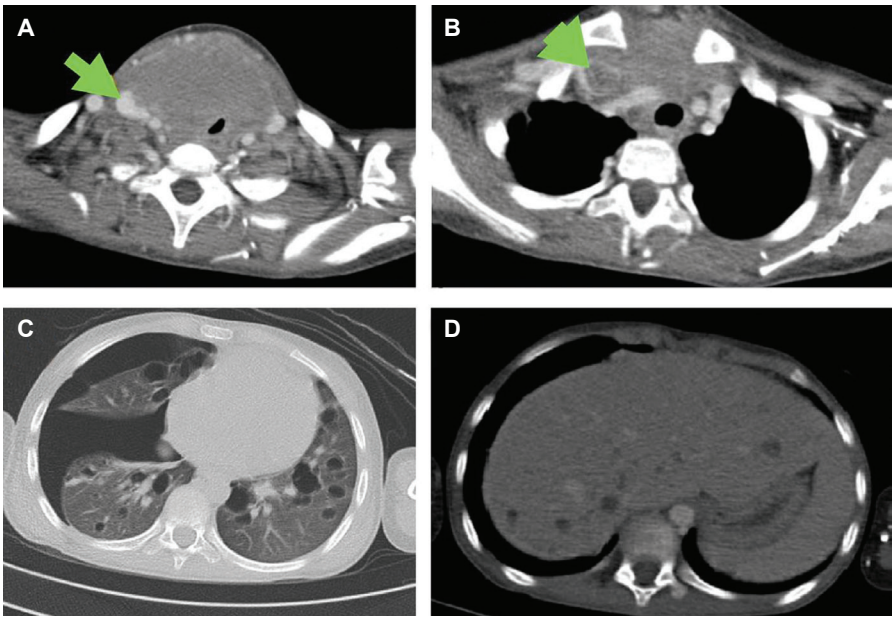

Figure 2. CT scan showing a heterogeneously large lesion replacing the normal looking thyroid lobes. The mass exhibits encroachment to the adjacent jugular veins **(A)** as well as the SVC **(B)**. There is also a pneumothorax along with multiple pulmonary bleb and bullae with thin septations **(C)** and hepatomegaly with multiple cysts **(D)**.

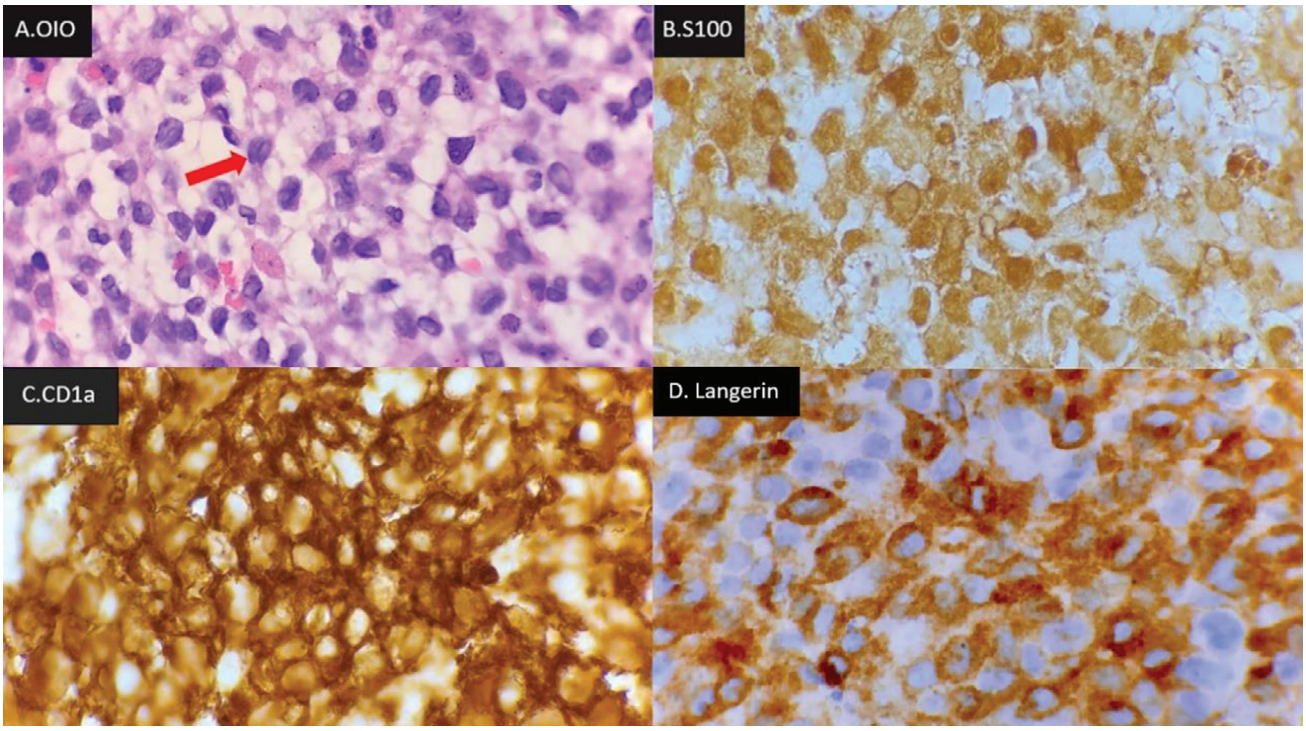

Figure 3. Photomicrographs of an oil power objective showing oval neoplastic cells with grooved nuclei *(red arrow)* with abundant amphophilic to eosinophilic cytoplasm **(A)**, S100 stain showing strong diffuse nuclear and cytoplasmic positivity **(B)**, CD1a stain showing strong diffuse membranous positivity **(C)**, and positive Langerin staining **(D)**.

DISCUSSION

LCH as a cause of goiter is rare, as the most common cause of goiter worldwide is iodine deficiency. Other causes may be natural goitrogens, smoking, autoimmune disorders, drugs rich in iodine, environmental agents, thyroid cancer and infiltrative diseases of the thyroid.[4,5]

LCH commonly presents as a tumor, skin rash, lytic bone lesions, pneumothorax, interstitial lung disease, or central diabetes insipidus. Microscopically, lesions consist of large histiocytes with abundant cytoplasm intermixed with lymphocytes and eosinophils.[6] In the "Activated-Immature model," LCH is thought to come from pathologic epidermal Langerhans cells that undergo malignant transformation or multiply because of immune dysregulation and form lesions in different organs. However, a newer proposed model called "The Misguided Myeloid Dendritic Cell Precursor Model," hypothesized that LCH lesions could come directly from bone-marrow-derived dendritic cell precursors which migrate to site of LCH lesions and differentiate into $CD207^+$ cells. Gene expression profiling of the pathologic cells seen in LCH showed that their profile is closer to immature dendritic cells than of epidermal Langerhans cell.[7] The dendritic cell precursors come from the bone marrow and the tropism of Langerhans cell is in the epidermis. This may explain why LCH is more common in the skeletal and integumentary system. The most common mutation identified in LCH is BRAF V600E, the presence of which correlates with multisystemic disease and poor survival.[8]

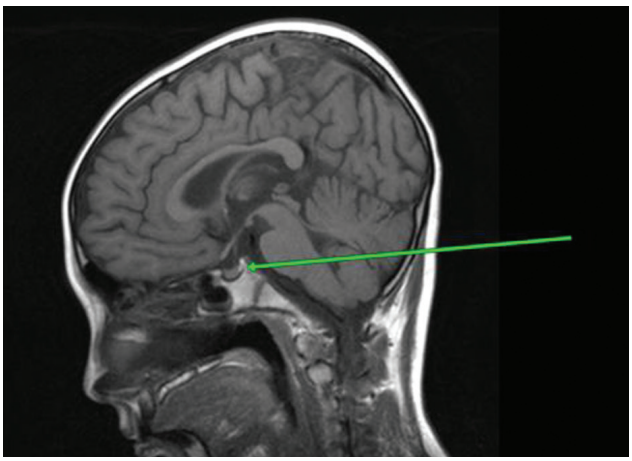

Figure 4. Midline sagittal T1 weighted image demonstrates the normal posterior pituitary bright spot.

LCH may develop in any tissue, but an incidence study showed that it most commonly affects bone, skin, and lymph nodes.[2] Seventy-five cases of adults and children with thyroid involvement in LCH have been reported. There were more cases in adults than in children (47 adults and 18 children),[9] and thyroid involvement is part of multisystemic LCH in the majority of cases; solitary involvement of the thyroid gland in LCH is extremely rare. It may present as goiter due to LCH infiltration in the thyroid.[10] Although the cause of thyroidal LCH is unclear, it is likely that encapsulation of the thyroid contributes to the rarity of its involvement. Proliferation of LCH in the thyroid often extends beyond the capsule which leads to its adherence to the surrounding structures.[11]

In the review of cases, a 38-year-old female presented with goiter and histopathology was consistent with LCH. Chest computed tomography showed bilateral multiple peripheral small nodules. Abdominal CT scan showed hepatomegaly with diffusely increased hepatic density.[12] Another 29-year-old female had LCH with thyroid, lung, and liver involvement. She also had diabetes insipidus, with polyuria being her chief complaint. She was treated with chemotherapy resulting to reduction in goiter size.[13]

When patients present with thyroid enlargement, thyroid function tests are part of the work up. Thyroid ultrasonography is especially important in those with palpable thyroid nodules, gland asymmetry, or suspicious cervical lymphadenopathy. Further evaluation with FNAC may be warranted depending on the size and ultrasound characteristics of thyroid lesion.[14]

In this patient, thyroid carcinoma was highly considered in the background of a rapidly enlarging neck mass associated with weight loss. FNAC showed a moderately cellular aspirate with abundant scattered large monomorphic atypical cells with increased nuclear-to-cytoplasmic ratio, scant cytoplasm, irregularly-shaped nuclei and inconspicuous nucleoli. The atypical cells form occasional microfollicles and are admixed lymphocytes in a background of colloid. During this time, a solitary thyroid lesion was considered, but the current involvement of the lungs and liver was not yet known.

The Bethesda System for reporting thyroid cytopathology favored category IV which is suspicious for follicular neoplasm. The recommendation for this lesion is surgical excision, but molecular testing may also be done to supplement risk assessment before doing surgery.[15] During admission, the patient had other manifestations such as pulmonary blebs and hepatic cysts, signs that the disease was not limited to the thyroid.

The diagnosis of thyroid LCH can be difficult. Fine needle aspiration can be useful in the diagnosis but Langerhans cells can still be misinterpreted as some other cells.[10] The diagnosis of LCH on FNAC may pose a challenge to the cytopathologist due to its rarity, low clinical index of suspicion, and overlapping cytologic findings in more common primary thyroid pathologies.[16] A similar case was published where the initial cytological interpretation of the thyroid FNA suggested a follicular neoplasm but histopathology after subtotal thyroidectomy revealed LCH. Thyroid LCH could be easily mistaken as undifferentiated carcinoma, lymphoma, lymphocytic thyroiditis, chronic granulomatous thyroiditis, and cystic degeneration of multinodular goiters. Immunohistochemical reactivity of histiocytes of S100 and CD1a is useful in diagnosing a case of LCH.[17]

LCH with thyroid involvement can be solitary or part of multisystem LCH. There are cases reported where the thyroid is the only organ involved in LCH.[16] Hence, in patients with a solitary goiter, LCH should also be included in its possible causes. Others report cases of LCH of the thyroid with a concomitant thyroid carcinoma.[18,19] In this patient, there was no evidence that a concomitant thyroid carcinoma exists.

LCH involving the thyroid gland may present with different states of thyroid dysfunction. In a review of sixty-six cases, majority are euthyroid (40.9%), followed by hypothyroid (19.7%), and less commonly, some have subclinical hypothyroidism (10.6%) or subclinical hyperthyroidism (1.5%).[9] This patient was initially euthyroid but developed subclinical hypothyroidism with the rapidly enlarging goiter. With prompt initiation of chemotherapy, thyroid function reverted to normal and prolonged levothyroxine treatment was avoided.

After confirming the diagnosis of thyroid LCH, work up for other systemic involvement should be done. Thoracic CT scan, abdominal ultrasonography, bone scan, and bone marrow aspiration can distinguish single organ involvement from multisystemic disease.[20] For this patient, a CT scan of the cranium and abdomen, along with bone marrow aspiration with biopsy ruled out cranial and bone marrow involvement, but confirmed the presence of cystic lesions in the lungs and liver.

Treatment depends on whether LCH is solitary or with multisystem involvement. For primary thyroid LCH, treatment options are surgery, chemotherapy or a combination of surgery with adjuvant chemotherapy.[9] Multisystemic LCH treatment includes vinblastine 6 mg/m^2 intravenously weekly bolus for 6 weeks and prednisone 40 mg/m^2/day given orally in three divided doses for 4 weeks which is then tapered over the next two weeks. Patients undergo reevaluation after the first 6 weeks of treatment, and treatment may be continued depending on the assessment.[21] Majority of children with thyroid LCH received combination of surgery and chemotherapy.[9] This patient with multisystemic LCH received chemotherapy based on the LCH-II study consisting of prednisone 40 mg/m^2/day and vinblastine 6 mg/m^2 intravenously weekly bolus for 6 weeks.

Prognosis of LCH depends on the presence of risk organ (i.e., bone marrow, liver, and spleen) involvement and response to initial systemic therapy. Patients with single-system disease have an excellent prognosis, with a survival rate of almost 100%, with a 5-year recurrence rate of <20%, usually involving the same organ system. On patients with risk organ involvement at diagnosis, the projected survival is 77%.[22] This patient had liver involvement but had good response to initial chemotherapy.

CONCLUSION

LCH as a cause of goiter is rare. In children, it is most commonly part of a multisystemic LCH rather than a single system. Being unaware that LCH may present as an anterior neck mass may lead to misdiagnosis. A high

index of suspicion and the help of a multidisciplinary team is needed for proper diagnosis and timely treatment.

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Ethical Consideration
Parental consent was obtained before the submission of the manuscript.

Statement of Authorship
All authors certified fulfillment of ICMJE authorship criteria.

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KMB: Data Curation, Writing - original draft preparation, Writing - review and editing, Visualization; **MJC:** Data Curation, Visualization; **CS:** Data Curation, Writing – review and editing, Visualization, Supervision, Project administration; **EF:** Data Curation, Visualization, Supervision; **MM:** Data Curation, Visualization, Supervision; **LA:** Data Curation, Writing – review and editing, Visualization, Supervision, Project administration.

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Written and Oral Examination for

Diplomate in Endocrinology, Diabetes and Metabolism

JANUARY 26, 2024 (Friday)
WRITTEN EXAMINATION

JANUARY 28, 2024 (Sunday)
ORAL EXAMINATION

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Journal of the
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THAN THAN AYE | Associate Editor | Prof. Aye has nothing to disclose.

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Subject: **SUBMISSION OF MANUSCRIPT FOR PUBLICATION**

We intend to publish the manuscript/, entitled "_____," under the Section *[Original Article, Review Article, Feature Article, Case Report, Case Series, Interhospital Grand Rounds, Brief Communications, Letter-to-the-Editor, Special Announcements]* in the Journal of the ASEAN Federation of Endocrine Societies.

LIST OF AUTHORS

Complete Name	Position/ Designation	Institutional Affiliation	Role in writing the manuscript	Email address	ORCID iD

On behalf of all the authors, I shall act as the corresponding author with the journal from this point onward.

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Gliclazide

Scored Tablets

YOUR TRUSTED PARTNER

Achieve the goal and maintain control[1]

▶ **UP TO 2 TABLETS DAILY, IN THE MORNING**

Indication: T2D in adults when dietary measures, physical exercise, and weight loss alone are not sufficient to control blood glucose.

1. Zaccardi F et al. *Diabetes Obes Metab.* 2020 Aug 5. Doi:10.1111/dom.14169

COMPOSITION* Diamicron 60 mg MR, modified release tablet containing 60 mg of gliclazide, contains lactose as an excipient. **INDICATION*** Non insulin-dependent diabetes (type 2) in adults when dietary measures, physical exercise and weight loss alone are not sufficient to control blood glucose. **DOSAGE AND ADMINISTRATION*** One half to 2 tablets per day i.e. from 30 to 120 mg taken orally as a single intake at breakfast time, including in elderly patients and those with mild to moderate renal insufficiency with careful patient monitoring. One tablet of Diamicron 60 mg MR is equivalent to 2 tablets of Diamicron 30 mg MR. The breakability of Diamicron 60 mg MR enables flexibility of dosing to be achieved. In patients at risk of hypoglycemia, daily starting dose of 30 mg is recommended. *Combination with other antidiabetics:* Diamicron 60 mg MR can be given in combination with biguanides, alpha glucosidase inhibitors or insulin (under close medical supervision). **CONTRAINDICATIONS*** Hypersensitivity to gliclazide or to any of the excipients, other sulfonylurea or sulphonamides; type 1 diabetes; diabetic pre-coma and coma, diabetic ketoacidosis; severe renal or hepatic insufficiency (in these cases the use of insulin is recommended); treatment with miconazole (see interactions section); lactation (see fertility, pregnancy and lactation section). **WARNINGS *** Hypoglycemia may occur with all sulfonylurea drugs, in cases of accidental overdose, when calorie or glucose intake is deficient, following prolonged or strenuous exercise and in patients with severe hepatic or renal impairment. Hospitalization and glucose administration for several days may be necessary. Patient should be informed of the importance of following dietary advice, of taking regular exercise and of regular monitoring of blood glucose levels. To be prescribed only in patients with regular food intake. Use with caution in patients with G6PD-deficiency. Excipients: contains lactose. **INTERACTION(S)*** Risk of hypoglycemia – *contraindicated:* miconazole; *not recommended:* phenylbutazone; alcohol; *use with caution:* other antidiabetic agents, beta-blockers, fluconazole, ACE inhibitors (captopril, enalapril), H2-receptor antagonists, MAOIs, sulfonamides, clarithromycin, NSAIDs. Risk of hyperglycaemia – *not recommended:* danazol; *use with caution:* chlorpromazine at high doses; glucocorticoids; ritodrine; salbutamol; terbutaline, Saint John's Wort (*hypericum perforatum*) preparations. Risk of dysglycaemia – *use with caution:* fluoroquinolones. Potentiation of anticoagulant therapy (e.g. warfarin), adjustment of the anticoagulant may be necessary. **PREGNANCY*:** Change to insulin before a pregnancy is attempted, or as soon as pregnancy is discovered. **BREASTFEEDING*:** contra-indicated. **FERTILITY* DRIVE & USE MACHINES*** Possible symptoms of hypoglycemia to be taken into account especially at the beginning of the treatment. **UNDESIRABLE EFFECTS*** Hypoglycemia, abdominal pain, nausea, vomiting, dyspepsia, diarrhea, constipation. Rare: changes in haematology generally reversible (anaemia, leucopenia, thrombocytopenia, granulocytopenia). Raised hepatic enzymes levels (AST, ALT, alkaline phosphatase), hepatitis (isolated reports). If cholestatic jaundice: discontinuation of treatment. Transient visual disturbances at start of treatment. More rarely: rash, pruritus, urticaria, angioedema, erythema, maculopapular rashes, bullous reactions such as Stevens-Johnson syndrome and toxic epidermal necrolysis and autoimmune bullous disorders, and exceptionally, drug rash with eosinophilia and systemic symptoms (DRESS). As for other sulfonylureas: observed cases of erythrocytopenia, agranulocytosis, haemolytic anaemia, pancytopenia, allergic vasculitis, hyponatraemia, elevated liver enzymes, impairment of liver function (cholestasis, jaundice) and hepatitis which led to life-threatening liver failure in isolated cases. **OVERDOSE*** Possible severe hypoglycemia requiring urgent IV glucose, immediate hospitalization and monitoring. **PROPERTIES*** Diamicron 60 mg MR is a sulfonylurea reducing blood glucose levels by stimulating insulin secretion from beta cells in the islets of Langerhans, thereby restoring the first peak of insulin secretion and increasing the second phase of insulin secretion in response to a meal or intake of glucose. Independent haemovascular properties. **PRESENTATION*** Box of 30, 90 or 100 tablets of Diamicron 60 mg MR in blister. **LES LABORATOIRES SERVIER**, 50 rue Carnot, 92284 Suresnes cedex France. **www.servier.com . *For complete information, please refer to the Summary of Product Characteristics for your country.**

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