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Sustainability: An Open Letter to our Authors and Readers

The Journal of the ASEAN Federation of Endocrine Societies (JAFES) stands at a crucial juncture as we navigate the evolving landscape of academic publishing. Our commitment to **open access** publishing has fostered a rich environment for enhancing research and collaboration. This accessibility is vital for advancing the field of endocrinology in our region, where disparities in healthcare resources often limit access to essential research findings. However, maintaining the quality and accessibility of our journal requires a sustainable financial model that supports our operational needs. The costs associated with editorial management, peer review, and online hosting are substantial.

JAFES' publisher, the seven country societies making up the Federation, have faithfully provided financial support to the journal, allowing the Philippine team to build capacity and to professionalize its operations. This support has enabled us to publish the journal without need to levy any expenses to authors who submit their work. We remain grateful to be given this privilege of hosting the JAFES and steering it to its current stature.

Sustainability is key as we move towards our 15th year of hosting the journal. This concern was discussed in length at a special session at last year's AFES Congress in Thailand, officiated by Endocrine Society of Thailand President Chaicharn Deerochanawong. (Figures 1 to 3).

To ensure that JAFES can continue to serve our authors, readers and the community effectively, we must introduce an Article Processing Charge (APC). This decision is not taken lightly; it stems from a commitment to uphold the integrity and quality of our

Figure 1. JAFES Core Team with MEMS and PCEDM Presidents at the AFES Presidents' Meeting in Queen Sirikit, Bangkok, Thailand on November 18, 2023 [from L-R: Gabriel V. Jasul Jr. (Associate Editor), Cecilia A. Jimeno (Vice Editor-in-Chief), Elizabeth Paz-Pacheco (Editor-in-Chief), Nurain Mohd Noor (MEMS President and JAFES Editorial Board Member), Marjorie A. Ramos (PCEDM President), Melissa Tandoc (JAFES Secretary)].

Figure 2. AFES Presidents [from R-L: Ketut Suastika (ISE), Nurain Mohd Noor (MEMS), Ko Ko (MSEM), Tran Huu Dang (VADE) Chaicharn Deerochanawong (EST), Marjorie A. Ramos (PCEDM), Kek Peng Chin (EMSS)], and JAFES [Elizabeth Paz-Pacheco (Editor-in-Chief), Gabriel V. Jasul Jr. (Associate Editor), Cecilia A. Jimeno (Vice Editor-in-Chief)], convene to discuss the journal's sustainability and future directions.

https://doi.org/10.15605/jafes.039.02.01

Figure 3. AFES Country Presidents and representatives following the meeting in Bangkok [from L-R: Tran Huu Dang (VADE President), Marjorie A. Ramos (PCEDM President), Vivien Lim (AFES 2022 President), Kek Peng Chin (EMSS President), Chaicharn Deerochanawong (AFES 2023 President, EST), Ko Ko (MSEM President), Ketut Suastika (past ISE President), Fatimah Eliana (ISE), Nurain Mohd Noor (MEMS), Roy Panusuan Sibarani (ISE)].

journal while ensuring that it remains open and accessible to all. The implementation of an APC will help cover operational expenses and provide the resources for continuous improvement in our publishing processes, including enhancing our editorial support, expanding our outreach, and investing in digital infrastructure.

At this juncture, the gradual introduction of an APC is a necessary step towards ensuring the long-term sustainability of JAFES. The details of the APC, including the specific fee structure and information on waivers or discounts for authors facing financial constraints, will be provided in the coming months. Our goal is to ensure that all researchers are able to continue to publish their work without barriers: this is JAFES' commitment.

As we move forward with this initiative, we invite dialogue with our authors, readers and other stakeholders. Your feedback is invaluable as we strive to create a model that balances sustainability with our mission to promote knowledge sharing and collaboration in the field of endocrinology.

Together, we can build a resilient platform that continues to advance endocrine research and practice. We appreciate your understanding and continued support as we implement this change. If you have any questions or concerns, please feel free to reach out to us.

Thank you for your commitment to advancing endocrinology research in our region.

Elizabeth Paz-Pacheco
Editor-in-Chief

Sydney Declaration on Predatory or Pseudo Journals and Publishers

We, the participants in the Joint Meeting of the Asia Pacific Association of Medical Journal Editors (APAME), the Western Pacific Region Index Medicus (WPRIM), and Index Medicus of the South-East Asia Region (IMSEAR), held in Newcastle, New South Wales, Australia from August 28 to 30, 2024:

CONSIDERING

That predatory (or pseudo) journals and publishers offer open access publication in exchange for fees without robust editorial or publishing services; these include "fake" or "scam" journals or publishers who send phishing emails which promise quick review;

That the articles collected by predatory (or pseudo) journals or publishers may never be published, or often are published with poor quality or accessibility, irrespective of any attempts by authors to withdraw them, resulting in such research effectively being lost;

CONFIRM

Our commitment to uphold the quality and integrity of our individual journals and their respective submission, editing and review processes, in opposition to predatory (or pseudo) journal practices;

Our commitment to exercise vigilance and safeguard the quality and integrity of our respective publishers against predatory (or pseudo) publication processes;

Our commitment to ensure that member journals of the Asia Pacific Association of Medical Journal Editors (including those indexed in the Western Pacific Region Index Medicus and Index Medicus of the South-East Asia Region) and their publishers do not engage in predatory (or pseudo) journal or publication practices;

CALL ON

Member States of and governments in the World Health Organization (WHO) Western Pacific and South-East Asia Regions, in collaboration with stakeholders from the nongovernmental and private sectors, to formulate and implement procedures and processes for identifying and dealing with predatory (and pseudo) Sydney Declaration on Predatory or Pseudo Journals and Publishers journals and publishers, and for guiding new and existing journals away from engaging in predatory (and pseudo) journal and publisher practices;

Stakeholders from the public and private sectors, national and international organizations, universities and academic societies to support WPRIM, IMSEAR, the Global Index Medicus of WHO, in ensuring the availability of high quality health information for all that is not marred by predatory (and pseudo) journal and publication practices;

COMMIT

Ourselves and our journals not to engage in predatory (or pseudo) journal practices, by learning about and implementing best journal practices, in accordance with the recommendations and guidelines issued by such bodies as the International Committee of Medical Journal Editors (ICMJE), the Committee on Publication Ethics (COPE), and the World Association of Medical Editors (WAME);

Our organization, APAME, to building collaborative networks, convening meaningful conferences, and organising participative events to educate and empower editors, peer reviewers, authors, librarians, and publishers to recognise and avoid engaging in predatory (or pseudo) journal and publisher practices.

30 August 2024, Newcastle, NSW, Australia

This declaration was launched at the 2024 Convention of the Asia Pacific Association of Medical Journal Editors (APAME) held in New South Wales, Australia from 28 to 30 August 2024. It is concurrently published in Journals linked to APAME and listed in the Index Medicus of the South-East Asia Region (IMSEAR) and the Western Pacific Region (WPRIM).

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EARLY-BIRD REGISTRATION DEADLINE EXTENDED!

31 JANUARY 2025

	Early Bird Before 31 January 2025	Regular 1-14 February 2025	Onsite From 15 February 2025
Main Conference			
Profession	7,000 THB	8,000 THB	9,000 THB
Student	3,500 THB	4,000 THB	5,000 THB

Note: Student registration must be accompanied by student ID card or letter from head of the department confirming his/her studying status.

Workshop 1

Technology enhanced learning:

Note: Participants have to bring your own laptops.

Workshop 1A 09:00 – 12:00 hrs. – Generative AI for 10x Productivity
Workshop 1B 13:30 – 16:30 hrs. – The Future of Digital Media

Workshop 2

Workshop 2 09.00-16.30 hrs. – The Art and Science of Clinical Reasoning: A Holistic Approach to Learning

Workshop 3

Workshop 3A 09.00 – 12.00 hrs. – Medical Educational Research: How to conduct?
Workshop 3B 13.30 – 16.30 hrs. – ASPIRE Academy: Key Ingredient in Promoting Student Engagement in Undergraduate Medical Education

Workshop	Early Bird	Regular	Onsite	
1A	1,500 THB	2,000 THB	2,500 THB	No Lunch
1B				
3A				
3B				
2	2,500 THB	3,000 THB	3,500 THB	With Lunch
1A + 1B				
1A + 3B				
3A + 1B				
3A + 3B				

JUNE 25 2024 — REGISTRATION OPENS

JUNE 30 2024 — ABSTRACT SUBMISSION OPENS

NOVEMBER 30 2024 — ABSTRACT SUBMISSION DEADLINE

JANUARY 31 2025 — EARLY BIRD REGISTRATION DEADLINE

FEBRUARY 14 2025 — REGULAR REGISTRATION DEADLINE

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REGISTRATION

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ORIGINAL ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

Correlation between Waist Circumference and IGF-1 Levels in an Elderly Population in Bali, Indonesia

I Made Pande Dwipayana,[1] Wira Gotera,[1] Made Ratna Saraswati,[1] I Made Siswadi Semadi,[1] Ida Bagus Aditya Nugraha,[1] Ketut Suastika,[1] Anak Agung Gede Budhiarta,[1] Padma Amrita,[2] Wahyu Pratama Putra[2]

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Abstract

Background. Hyperinsulinemia due to insulin resistance is hypothesized to act as a promotor of cancer growth. In addition to the direct effects of hyperinsulinemia on cancer cells, the stimulation of tumor cell growth can also be indirectly mediated through growth factors and receptors such as insulin-like growth factor 1 (IGF-1). Increased cancer risk is also associated with increased adipose tissue, such as in abdominal obesity, due to the higher risk of insulin resistance and hyperinsulinemia. Waist circumference is a parameter that indicates an individual's level of adiposity. In addition, the risk of cancer also increases in the elderly as they age. This study aims to assess the correlation between waist circumference and IGF-1 levels in the elderly population in Bali, Indonesia.

Methodology. This study used a cross-sectional analytical design conducted in the Melinggih Village, Gianyar Regency. The study was conducted in September 2023. This study has been approved by the Research Ethics Commission number 2020/UN14.2.2.VII.14/LT/2023. The study population included elderly individuals residing in the Melinggih Village who were willing to participate. Data analysis encompassed descriptive analysis and the Spearman correlation test.

Result. A total of 88 subjects participated in the study, consisting of 57 females (64.8%) and 31 males (35.2%). A statistically significant but weak correlation coexists between waist circumference and IGF-1 levels.

Conclusion. A weak but statistically significant positive correlation was found between waist circumference and IGF-1 levels in the elderly. However, because of the small sample size, another study with a bigger sample size with enough power to investigate this association needs to be done to validate the results of the current study.

Key words: elderly, IGF-1, waist circumference

INTRODUCTION

Hyperinsulinemia due to insulin resistance is hypothesized to act as a promotor of cancer growth. Moreover, hyperinsulinemia has been implicated etiologically in carcinogenesis. In individuals with long-standing diabetes mellitus (DM), pancreatic beta cells produce endogenous insulin at lower levels than those who are prediabetic. This finding aligns with research indicating a reduced risk of colorectal cancer with a longer duration of DM. A meta-analysis revealed that the risk of colorectal cancer with DM duration less than ten years was 1.3 times, while a duration greater than ten years posed a risk of 1.17 times.[1]

In addition to the direct effects of insulin on cancer cells, the promotion of tumor cell growth could also be indirectly mediated through growth factors such as insulin-like growth factor 1 (IGF-1). Elevated insulin concentrations reduce the levels of IGF-binding protein-1, subsequently increasing the amount of bioactive IGF-1. IGF-1 exhibits mitogenic and anti-apoptotic activities and may function as a stimulus for preneoplastic and neoplastic growth. Patients with bladder cancer were also found to have elevated plasma levels of IGF-1.[2,3]

Beyond the direct effects of insulin, adiposity was also associated with increased cancer risk. Adipose is an

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endocrine-active tissue that produces free fatty acids, interleukin-6 (IL-6), monocyte chemotactic protein, plasminogen activator inhibitor-1 (PAI-1), adiponectin, leptin and tumor necrosis factor-α (TNF-α).[4,5]

Waist circumference is one parameter indicating an individual's level of adiposity. In the Asian population, waist circumference is also a parameter for the diagnosis of abdominal obesity. In Indonesia, we use body mass index (BMI) as a criterion. A BMI ≥23.0 kg/m^2 is classified as overweight, a BMI between 25 to 29.9 kg/m^2 is obese grade 1, and a BMI ≥ 30.0 kg/m^2 is obese grade 2.[6] Cancer risk also increases in the obese and elderly population. Although abdominal obesity is a protective factor that plays a role in breast tumors in women aged 25 to 64 in Indonesia,[7] the burden of cancer attributable to obesity is mainly related to digestive organs, with high prevalence in the female population.[8] Commonly cited cases are colon cancer (23,051), ovarian cancer (21,911) and pancreatic cancer (4,564). A waist circumference (WC) of ≤80 cm was linked to a 78% breast cancer risk reduction.[9] The study aims to examine the correlation between waist circumference and IGF-1 levels in the elderly in Bali, Indonesia.

This research seeks to investigate metabolic disorders, particularly obesity as a component of metabolic diseases, and its relation to cancer risk. It aims to provide data on the relationship between waist circumference and IGF-1 levels in the elderly and its possible role in cancer risk. This research is also expected to serve as a foundation for further studies. Mainly, the objective of this study is to elucidate the correlation between waist circumference and IGF-1 levels in the elderly.

METHODOLOGY

This study employed an analytical cross-sectional research design conducted in Melinggih Village, Gianyar Regency. The research was conducted in September 2023. This study has been approved by the Research Ethics Commission number 2020/UN14.2.2.VII.14/LT/2023. Elderly people (aged 60 and above) from Melinggih Village were invited to participate in this study. Those who provided informed consent, either verbally or in writing, were included. Individuals with a history of malignancy and liver disease were excluded from the research. Consequently, a total sample of 88 individuals was recruited, and their IGF-1 levels were measured using the human Insulin-like Growth Factor-1 ELISA Kit (IGF1) (ab108873) with the double antibody sandwich method produced by Yanaihara Institute Inc. (Multispecies specificity), Cat. No.: RSCYK160R. The examination results were expressed numerically, in ng/mL units.

Descriptive statistics were used to describe the characteristics of the survey respondents. Quantitative data was examined using the Shapiro-Wilk test, while the mean and standard deviation or the median and range were used to summarize the data. Categorical data is described using frequency and percentage. Spearman's rank correlation was used to determine the correlation between IGF-1 levels and the following: waist circumference, body mass index and waist-hip ratio using the IBM SPSS Statistics version 26.0 program.

RESULTS

Eighty-eight elderly patients from Melinggih Village participated in this study, with a median age of 68.50 years, ranging from 60 to 84. Most participants were female, accounting for 64.8% (n = 57), while males comprised 35.2% (n = 31). Median BMI was 22.50 kg/m² (14.60 to 34.30 kg/m²). It consists of 8.0% (n = 7) underweight, 45.5% (n = 40) normal weight, 13.6% (n = 12) overweight, 26.1% (n = 23) obesity grade 1, 6.8% (n = 6) obesity grade 2.

Meanwhile, the mean waist circumference was 84.50 cm with SD of 10.42 cm overall, with females having an average waist circumference of 84.80 cm (SD = 11.45 cm) and males having an average of 84.16 cm (SD = 8.35 cm). In addition, 61 patients (69.3%) had no central obesity, and 27 patients (30.7%) had central obesity.

Blood pressure readings indicated an average systolic blood pressure of 149.03 mm Hg (SD = 25 mm Hg) and an average diastolic blood pressure of 85.62 mm Hg (SD = 14.99 mm Hg). Mean IGF-1 level of the participants was 4.35 ng/mL (SD = 2.56 ng/mL). The demographic and clinical characteristics of the participants are detailed in Table 1.

There is a statistically significant but weakly positive correlation between the waist circumference and IGF-1 levels (r = 0.255, P = 0.016) (Table 2).

Table 1. Demographic and clinical characteristics of the study participants (N = 88)

Characteristics	Mean ± SD
Age (years), median (min-max)	68.50 (60 - 84)
Sex, n (%)	
Female	57 (64.8)
Male	31 (35.2)
Height (cm)	152.42 ± 6.53
BMI (kg/m^2), median (min-max)	22.50 (14.60 – 34.30)
Body weight (kg)	53.20 ± 10.59
Waist circumference, overall (cm)	84.50 ± 10.42
Waist circumference, females (cm)	84.80 ± 11.45
Waist circumference, males (cm)	84.16 ± 8.35
Systolic blood pressure (mm Hg)	149.03 ± 25
Diastolic blood pressure (mm Hg)	85.62 ± 14.99
IGF-1 level (ng/ml)	4.35 ± 2.56

BMI: Body Mass Index, IGF-1: Insulin Like Growth Factor-1

Table 2. Correlation between select clinical factors and IGF-1 Level

Clinical Factors	r	*p*-value
Waist circumference and IGF-1	0.255	0.016*
Male	0.366	0.043*
Female	0.222	0.097
Body mass index and IGF-1	0.284	0.007**
Waist-hip ratio and IGF-1	0.062	0.565

*p <0.05, **p <0.01, ***p <0.001

When this correlation was determined by sex, a statistically significant but weakly positive correlation was also found in males (r = 0.366, *P* = 0.043), while in females, the correlation was not statistically significant (r = 0.222, *P* = 0.097).

The correlation of IGF-1 levels with BMI and waist-hip ratio was also examined. BMI also had a significant weak positive correlation with IGF-1 levels (r = 0.284, *P* = 0.007), while its correlation with waist-hip ratio was not statistically significant (r = 0.062, *P* = 0.565).

DISCUSSION

Obesity is a chronic disease characterized by a pathophysiological process leading to an increase in adipose tissue mass, consequently increasing morbidity and mortality risk. Numerous conditions facilitate interactions between environmental factors and weight-regulating genes, resulting in a substantial portion of the population having a body mass index (BMI) ≥25 kg/m^2, associated with various metabolic complications of metabolic.[10]

The prevalence of obesity has surged in the last three decades, transforming it into a crucial issue that demands particular attention. Obesity is linked to reduced life expectancy and a higher risk of developing type 2 diabetes mellitus (T2DM), cardiovascular diseases and malignancies. Nevertheless, not all individuals with obesity are at high risk of mortality, indicating the existence of a subgroup known as metabolically healthy obese (MHO). MHO individuals do not present with typical metabolic abnormalities like dyslipidaemia, insulin resistance and hypertension. Additionally, there is a subgroup with normal weight but with metabolic abnormalities, termed metabolically obese normal weight (MONW). On the contrary, some individuals with obesity present with one or several metabolic disorders, identified as unhealthy metabolically obese (UMO).[11]

This study observed a statistically significant positive correlation between waist circumference and IGF-1 levels in the elderly. After we did the subgroup analysis, we also found a statistically significant positive correlation in male patients. We also analyzed BMI and IGF-1 levels, Waist-Hip Ratio and IGF-1 levels and found a statistically significant positive correlation in the former. Increased waist circumference signifies the accumulation of visceral fat, triggering insulin resistance. Insulin resistance gives rise to hyperinsulinemia. Hyperinsulinemia lowers the level of IGF binding protein-1, subsequently increasing levels of bioactive IGF-1. IGF-1 demonstrates mitogenic and anti-apoptotic activities, stimulating preneoplastic and neoplastic growth and forming the basis for elevated cancer risk. Cancer risk rises in the elderly population.[11]

This aligns with the findings of a meta-analysis by Lin et al. (2020), indicating that individuals with metabolically healthy obesity have a higher risk of cancer compared to those with metabolically healthy non-obesity (OR 1.14; 95% CI 1.05-1.23).[12] Conversely, a meta-analysis conducted by Zheng et al. (2022) found that MHO individuals have a lower cancer risk compared to those with metabolically unhealthy obesity (OR 0.71; 95% CI [0.61,0.84]).[13]

We were unable to analyze other possible confounders affecting IGF-1. We did not examine or measure the insulin resistance (HOMA-IR) due to a lack of resources. However, there are established studies that have reviewed the relationship between WHR and insulin resistance.

This study's limitation arises from the small sample size, which reduces the analytical power. This is also an observational study that cannot prove cause and effect. It is advised to avoid dichotomizing, which eventually reduces power and offers no additional benefits, optimize the power of creating confirmatory experiments centered around important topics and utilize targeted hypothesis testing.

CONCLUSION

A weak but statistically significant positive correlation was found between waist circumference and IGF-1 levels in this elderly population in a village in Bali, Indonesia. However, because of the small sample size, another study with a bigger sample size and enough power to investigate this association needs to be conducted to validate the results of the current study. Based on these research outcomes, the elderly should maintain a healthy lifestyle to prevent an increase in waist circumference as they age.

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The authors express their gratitude to the research team at Prof. dr. I Goesti Ngoerah Gde Ngoerah Hospital in Denpasar, Bali, for their diligent work in collecting, documenting, and analyzing the data.

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

Credit Author Statement
IMPD: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **WG:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **MRS:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **IMSS:** Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **IBAN:** Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **KS:** Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **AAGB:** Data Curation, Writing – original

draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **PA:** Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **WPP:** Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition.

Author Disclosure

The authors declared no conflict of interest.

Data Availability Statement

Datasets analyzed in the study are under license and not publicly available for sharing.

Funding Source

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ORIGINAL ARTICLE

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Endothelial Dysfunction Using Flow-mediated Dilatation Among Individuals with Pre-impaired Glucose Tolerance (Pre-IGT)

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Abstract

Objectives. Pre-impaired glucose tolerance (pre-IGT) is a prediabetes stage characterized by normoglycemia and compensatory hyperinsulinemia due to insulin resistance. Hyperinsulinemia increases cardiovascular disease (CVD) risk, especially, endothelial dysfunction (ED). However, there is paucity of studies on ED with hyperinsulinemia alone, particularly in individuals with pre-IGT. This study aimed to determine the presence of ED using brachial artery flow-mediated dilatation (FMD) among adult participants with pre-IGT and its correlation with insulin levels and other related clinical parameters.

Methodology. This is a cross-sectional analytical study. We screened adult patients with risk factors for developing diabetes (first-degree relative with type 2 diabetes mellitus, obesity, history of gestational diabetes and polycystic ovary syndrome). Brachial artery FMD was performed among participants with pre-IGT and findings were correlated with CVD risk factors using Pearson's correlation and linear regression.

Results. Of the 23 pre-IGT patients, 5 (21.74%) had decreased FMD values with significant associations with serum insulin and HbA1c. It was further observed that for every 1-unit increase in second-hour serum insulin and in HbA1c, there was a decrease in FMD values by 0.38% and 0.50%, respectively. Serum insulin was elevated, while other biochemical parameters were normal. Moreover, participants with low FMD were older, with higher BMI and had higher HBA1c, total cholesterol and low-density lipoprotein (LDL) cholesterol.

Conclusion. As early as the pre-IGT stage, endothelial dysfunction using the FMD test is already present, with red flags on other CVD risk factors already developing.

Key words: endothelial dysfunction (ED), insulin resistance (IR), pre-impaired glucose tolerance (Pre-IGT), hyperinsulinemia, type 2 diabetes mellitus (T2D), cardiovascular disease (CVD) risks

INTRODUCTION

Insulin resistance (IR) is the earliest metabolic abnormality in the pathophysiology of type 2 diabetes mellitus (T2D).[1] To overcome this, the pancreatic β-cells increase insulin secretion to maintain normal blood glucose, resulting in hyperinsulinemia.[1-5] Hyperinsulinemia in IR increases levels of C-peptide, a cleavage product of pro-insulin, which has proatherogenic effects.[6] Neointimal smooth muscle cell proliferation and vascular morphological modification have been attributed to the inflammatory effects of C-peptide lodging in the vascular intima-media of T2D patients.[7,8] The circulating C-peptide levels have also been associated with subclinical myocardial injury development.[9] The resultant vascular functional alteration, known as endothelial dysfunction (ED), which is identified through arterial intima-media thickness and abnormal arterial endothelial flow-mediated dilatation (FMD), has been elaborated in long-term adverse cardiovascular disease (CVD) outcomes and the morbidity and mortality of high-risk individuals with IR and hyperinsulinemia.[10-14]

Although these vascular abnormalities were implicated in the prediabetes stage like impaired glucose tolerance (IGT), most studies consistently implied abnormal circulating blood glucose (BG) as the primary causative factor for CVD development, compounded with major CVD risk factors like hypertension, dyslipidemia and smoking.[11-14]

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Nevertheless, there is a paucity of evidence on functional vascular abnormality or endothelial dysfunction using the pre-IGT stage as a clinical model, especially in the absence of significant risk factors.

Historically, in 1975, Kraft proposed the term *diabetes mellitus in situ* to characterize patients with normal glucose but abnormal insulin tolerance.[2] In elaborating on the natural course of T2D, de Groot and Jameson labeled this stage as compensated hyperinsulinemia.[5] Cognizant that the process of compensatory hyperinsulinemia with normoglycemia precedes the stage of IGT, our group termed this prediabetes stage as *pre-impaired glucose tolerance* (pre-IGT).[15-17] This preliminary study was conducted to determine vascular functional abnormality or endothelial dysfunction (ED), using brachial artery FMD, among adult participants with pre-IGT (hyperinsulinemic and normoglycemic) and its correlation with insulin levels and other related clinical parameters.

METHODS

Research design, study participants, and sample size

We conducted a cross-sectional, preliminary, small-scale study among adult patients at risk of developing T2D [first-degree relative with T2D, history of gestational DM (GDM), polycystic ovary syndrome (PCOS), overweight or obese and acanthosis nigricans].[18] Sixty-seven individuals aged 18 to 40 years old were purposively recruited at the Ambulatory Care Services of the University of Santo Tomas Hospital (Manila, Philippines). The World Health Organization Asian Body Mass Index (BMI) classification was used to categorize BMI (overweight: BMI = 23.00 to 24.90 kg/m^2, obese: BMI ≥25 kg/m^2).[19] We excluded patients diagnosed with T1D or T2D, IGT, impaired fasting glucose, hypertension (blood pressure >140/90 mm Hg), smoking history (either previous or current), dyslipidemia, coronary artery disease, cerebrovascular disease, chronic heart failure, liver disease and steroid intake for over a month in the past 3 months.[20]

Sample size (*priori*) computation was performed using the formula recommended by Viechtbaurer et al., for pilot or small-scale studies.[21] From the study of Skaug et al., the prevalence of endothelial dysfunction among low-risk healthy patients was 16.00%.[22] Using the recommended formula, a prevalence of 16.00%, and a significance level of 0.05 or 95% confidence interval, the computed sample size was 18 participants.

Written informed consent was obtained from all participants. This study was conducted under the Declaration of Helsinki and was approved by the University of Santo Tomas Hospital Research Ethics Committee (REC) (Reference No. REC-2021-05-070-TF). The manuscript is in line with the International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals.

Anthropometric and biochemical data

We measured BMI, systolic BP [normal value (NV) < 120 mm Hg] and diastolic BP (NV <80 mm Hg). All participants underwent the following biochemical tests with their corresponding NV: fasting BG (< 100 mg/dL); post-75-g oral glucose tolerance test (OGTT) 2-hour BG (<140 mg/dL); 2-hour serum insulin (<30 uIU/mL);[23,24] HbA1c (< 5.7%), lipid profile [total cholesterol (<200 mg/dL), triglycerides (<150 mg/dL), low-density lipoprotein (LDL) cholesterol (<130 mg/dL), high-density lipoprotein (HDL) cholesterol (>40 mg/dL)][25]; creatinine (0.5 to 0.95 mg/dL); and alanine transaminase (ALT) (10 to 35 U/L). Those with normal fasting and 2nd-hour BG but with an elevated 2nd-hour insulin level were classified as pre-IGT.[15-17] The pre-IGT participants with generally normal biochemical results were included in the study (N = 23).

Brachial artery flow-mediated dilatation (FMD)

Included participants with pre-IGT subsequently underwent brachial artery FMD test. The following were done since several variables such as food, medication, temperature and sympathetic stimulation can affect FMD: (a) The participants were asked to fast for 8 to 12 hours before the procedure; (b) The procedure was performed at the University of Santo Tomas Hospital Heart Station, which is a quiet and temperature-controlled room; (c) The participants were asked not to exercise before the procedure; and (d) They were asked to avoid ingesting caffeine, high-fat foods and vitamin C for at least 4 to 6 hours before the procedure.[26]

A single technician did the procedure using ultrasonography (Philips CX50 model) with a 3-12 MHz linear probe (Philips L12-3). The measurements were obtained on the non-dominant arm while the participant was supine, allowing 20 minutes of rest before the procedure. Hyperemia was induced by inflation of a pneumatic cuff (12.5 cm wide) at 230–250 mm Hg for 5 minutes on the most proximal portion of the upper arm. The arterial diameter measurement was repeated 45–60 seconds after sudden cuff deflation. We utilized the average of the 3 measurements for the analysis. We calculated FMD as the percentage increase in diameter from the baseline to the maximum value after the cuff deflation. Interpretation of FMD results was performed by a vascular specialist who was blinded from the participants' characteristics. A positive result for endothelial dysfunction (ED+) was present if FMD values were less than 10% of the baseline diameter.[26] According to Kuvin et al., this cut-off score has a sensitivity of 91% and a negative predictive value of 95%. However, specificity was at 54% with a positive predictive value of 39%.[27]

Statistical analysis

Statistical analyses were conducted using STATA Statistical Software, Version 13, College Station, TX: StataCorp LP. A *p*-value of 0.05 was considered statistically significant.

Descriptive statistics included mean and standard and frequency and proportion for nominal data. Shapiro-Wilk's test was employed to determine data normality. Comparative analyses of the demographic and clinical characteristics according to ED status [without ED (ED-) vs. ED+)] were conducted using Chi-Square Test or Fisher's Exact test, if the assumption of at least 5 observations per cell is not met, for categorical variables; and Mann-Whitney U Test for ordinal and non-normally distributed, continuous data. Correlation analyses, using Pearson's Correlation, were initially utilized to determine the association of FMD with clinical parameters. Afterward, clinical parameters with significant associations were regressed with FMD values using linear regression. Crude analyses were initially performed and were adjusted to control for the confounders (age, sex, duration of being overweight or obese) using a 10% change in criterion estimate.

RESULTS

Demographics and clinical profiles

Among the 67 patients screened in this study, 17 were normal (normoglycemic and normoinsulinemic), 41 had pre-IGT (normoglycemic and hyperinsulinemic), 8 had IGT, and 1 had T2D. Of the 41 patients with pre-IGT, 23 were eligible for this study. We excluded 16 patients who had dyslipidemia (total cholesterol >200 mg/dL) and another 2 patients with elevated ALT.

As shown in Table 1, the mean age of participants with pre-IGT was 26.17 years (SD = 4.80). The majority were female (82.61%) and had a family history of T2D (73.91%). The mean BMI was 24.65 kg/m^2 (SD = 3.59). The mean OGTT BG at fasting and 2-hour post-load were both normal at 85.98 mg/dL (SD = 7.03) and 104.33 mg/dL (SD = 15.54), respectively. Likewise, the mean HbA1c was 5.25% (SD = 0.38), which was normal. The mean insulin at 2-hour was 109.90 uIU/mL (SD = 108.25). All participants were normotensive (SBP: 107.78 ± 11.95 mm Hg, range: 90-120 mm Hg; DBP: 71.48 ± 9.83 mm Hg, range: 60-80 mm Hg) and normolipidemic (total cholesterol: 170.76 mg/dL ± 17.83, range:143.24-199.61 mg/dL; triglycerides: 91.54 ± 55.13 mg/dL, range:34.52-145.14 mg/dL; LDL cholesterol: 95.03 ± 17.14 mg/dL, range: 67.57-129.73 mg/dL; HDL cholesterol: 57.36 ± 12.32 mg/dL, range: 40.00-85.71 mg/dL). Results also showed that the mean serum creatinine was 0.71 mg/dL (SD = 0.15) and the mean ALT was 24.95 U/L (SD = 32.81), which were both normal. Comparative analyses indicated that none of the demographic and clinical characteristics were significantly different between those with ED+ and ED– endothelial dysfunction (p >0.05). None had a history of GDM.

Table 1. Demographic and clinical profile of participants with pre-IGT according to endothelial dysfunction status (N = 23)

Characteristics	Endothelial dysfunction status[a]			Test statistic[b]	*p*-value (two-tailed)
	Without endothelial dysfunction or FMD ≥10% (n = 18)	With endothelial dysfunction or FMD <10% (n = 5)	Total (n = 23)		
Age (Years; x̄, SD)	25.83 (4.99)	27.40 (4.34)	26.17 (4.80)	0.48	0.502
Sex (f, %)				0.03	1.000
Male	3 (16.67%)	1 (20.00%)	4 (17.39%)		
Female	15 (83.33%)	4 (80.00%)	19 (82.61%)		
Family history of diabetes mellitus (f, %)	13 (72.22%)	4 (80.00%)	17 (73.91%)	0.12	1.000
Body mass index (kg/m^2; x̄, SD)	24.37 (3.34)	25.65 (4.69)	24.65 (3.59)	0.46	0.491
CVD risk factors (x̄, SD)					
Oral Glucose Tolerance Test (OGTT; mg/dL)					
Fasting blood glucose	85.93 (6.86)	86.17 (8.44)	85.98 (7.03)	0.88	0.914
2-hours post-OGTT	103.06 (15.90)	108.88 (14.86)	104.33 (15.54)	0.43	0.455
HbA1c (%)	5.21 (0.39)	5.38 (0.33)	5.25 (0.38)	0.33	0.352
2-hours insulin post-OGTT (uIU/mL)	92.21 (97.60)	173.58 (132.21)	109.90 (108.25)	0.30	0.325
Other CVD risk factors (x̄, SD)					
Systolic blood pressure (mm Hg)	108.50 (12.55)	105.20 (10.26)	107.78 (11.95)	0.53	0.633
Diastolic blood pressure (mm Hg)	72.22 (9.98)	68.80 (9.86)	71.48 (9.83)	0.54	0.614
Other biochemical tests (x̄, SD)					
Total cholesterol (mg/dL)	170.33 (18.65)	172.28 (16.28)	170.76 (17.82)	0.97	0.982
Triglyceride (mg/dL)	94.75 (59.74)	80.01 (36.51)	91.54 (55.13)	0.71	0.732
LDL-C (mg/dL)	93.59 (18.27)	100.23 (12.38)	95.03 (17.14)	0.31	0.331
HDL-C (mg/dL)	57.70 (12.56)	56.14 (12.73)	57.36 (12.32)	0.77	0.790
Serum Creatinine (mg/dL)	0.70 (0.12)	0.77 (0.22)	0.71 (0.15)	0.30	0.316
ALT (U/L)	24.73 (12.88)	25.72 (25.01)	24.95 (15.56)	0.46	0.491
Related CVD risk factors					
Duration of being overweight or obese (Years; x̄, SD)	5.33 (3.34)	3.60 (1.82)	4.96 (3.13)	0.21	0.232
Duration of PCOS (Months; x̄, SD)	13.13 (37.42)	0.25 (0.50)	10.42 (33.44)	0.59	0.664
History of gestational diabetes mellitus (f, %)	0 (0.00%)	0 (0.00%)	0 (0.00%)	–	–

Abbreviations and/or Symbols: x̄ = Mean, SD = Standard Deviation; f = Frequency, % = Percentage, CVD = Cardiovascular Disease, LDL = Low Density Lipoprotein, HDL = High Density Lipoprotein, ALT = Alanine Aminotransferase, PCOS = Polycystic Ovary Syndrome

[a]Note: FMD <10% denotes positive for endothelial dysfunction (ED+), while FMD >10% indicates negative for endothelial dysfunction (ED–)

[b]Note: Comparisons were conducted using Chi-Square Test or Fisher's Exact Test, for categorical variables, and Mann-Whitney U Test, for ordinal and non-normally-distributed continuous variables.

*Significant at 0.05

Table 2. Comparison of flow-mediated dilatation (FMD) among participants with pre-IGT according to endothelial dysfunction status (N = 23)

Characteristics	Endothelial dysfunction status			z-value[a]	*p*-value (two-tailed)
	Without endothelial dysfunction or FMD ≥10% (n = 18)	With endothelial dysfunction or FMD <10% (n = 5)	Total (n = 23)		
Flow-mediated dilatation score (x̄, SD)	25.25 (18.93)	6.34 (2.83)	21.14 (18.49)	3.35*	0.001

Abbreviations and/or Symbols: x̄=Mean, SD=Standard Deviation
[a]Note: Comparison was conducted using Mann-Whitney U Test.
*Significant at 0.05

As expected among pre-IGT participants, serum insulin was elevated with all other biochemical parameters within normal levels (Table 1). Although statistically not significant, comparative analyses showed that participants with low FMD (FMD <10% or ED+) were older, had higher BMI and higher fasting and 2-hour BG, HbA1c, total cholesterol and LDL cholesterol.

Endothelial dysfunction through FMD measurement

Among the 23 participants with pre-IGT who had brachial FMD test, 5 had low FMD <10% or ED+ signifying endothelial dysfunction (with a prevalence of 21.74% (95% CI: 7.46% to 43.70%). The FMD values of pre-IGT participants with FMD <10% (ED+) and FMD ≥ 10% (ED–) were 6.34% (SD: 2.83) and 25.25% (SD: 18.93), respectively (Table 2). Comparative analysis using the Mann-Whitney U Test showed that the FMD score of ED+ participants was significantly lower ($z = 3.35$, $p = 0.001$) than ED– participants.

Associations of FMD with clinical parameters

A significant, negative correlation of FMD score, albeit weak, was found with the 2-hour serum insulin ($r = –0.37$, $p = 0.048$) and HbA1c ($r = –0.48$ $p = 0.020$) (Table 3). Although insignificant, negative correlations with FMD were obtained with the following cardiometabolic parameters: fasting and 2-hour BG post-OGTT and duration of being overweight or obese.

Table 3. Correlation analyses using Pearson's R of the associations of flow-mediated dilatation (FMD) with different variables among patients with pre-IGT (N = 23)

Clinical parameters	FMD	
	r-value	*p*-value (two-tailed)
2-hour insulin post-OGTT (uIU/mL)	-0.37	0.048
HbA1c (%)	-0.48*	0.020
Fasting blood glucose (mg/dL)	-0.21	0.348
2-hour blood glucose post-OGTT (mg/dL)	-0.22	0.307
Body mass index (BMI; kg/m^2)	0.01	0.985
Duration of being overweight or obese (years)	-0.06	0.799
Status of polycystic ovary syndrome (PCOS)	0.20	0.423
Duration of PCOS (months)	0.13	0.595
Systolic blood pressure (mm Hg)	0.11	0.621
Diastolic blood pressure (mm Hg)	0.03	0.885

*Significant at 0.05

Results of the crude and adjusted linear regression analyses (Table 4) indicated that, after controlling for significant confounders (age, sex and duration of being overweight or obese), both 2-hour serum insulin ($\beta = –0.38$, $p = 0.050$) and HbA1c ($\beta = –0.50$, $p = 0.031$) had negative and significant associations with FMD. In particular, every 1-unit increase in 2-hour serum insulin leads to a 0.38% decrease in FMD values (1 unit of insulin is equivalent to 1 uIU/mL). Similarly, every 1-unit increase in HbA1c values denotes a 0.50% decrease in FMD values (1 unit of HbA1c is equivalent to 1%).

DISCUSSION

OGTT as a measure for IR and hyperinsulinemia

With OGTT, Stumvoll and colleagues demonstrated a high correlation between glucose metabolic clearance rate (MCR), insulin sensitivity index (ISI) and the first- and second-phase insulin release.[28] The group had shown that BMI, insulin (120 min) and glucose (90 min) were highly correlated with MCR ($r = 0.80$, $p < 0.00005$) and ISI ($r = 0.79$, $p < 0.00005$). Interestingly, the parameters predicted by the equations correlated better with the measured parameters than homeostasis model assessment (HOMA-IR) for secretion and resistance, the delta 30-min insulin/delta 30-min glucose ratio for secretion and insulin (120 min) for insulin resistance taken from the OGTT.

In the study by Crofts et al., over half of the 4,185 participants with normal glucose tolerance showed hyperinsulinemia after 75-g OGTT. In their report, however, fasting insulin had limited value in diagnosing hyperinsulinemia.[23] In their follow-up study, a 2-hour insulin level post-OGTT >30 uU/mL had the highest sensitivity of 98% in predicting

Table 4. Crude and adjusted analyses using linear regression of the association of the insulin at 2-hours post-prandial and HbA1c with flow-mediated dilatation of the participants with pre-IGT (N = 23)

Risk factors	Flow-mediated dilatation (FMD)					
	Crude or unadjusted analyses			Adjusted analyses		
	Crude β coefficient	Standard error (SE)	*p*-value (two-tailed)	Crude β coefficient	Standard error (SE)	*p*-value (two-tailed)
Insulin 2 hours	–0.37*	0.03	0.048	–0.38*	0.04	0.050
HbA1c	–0.48*	9.42	0.020	–0.50*	10.46	0.031

[a]The beta coefficients were adjusted for the confounding effects of Age, Sex, and Duration of Body Mass Index.
*Significant at 0.05

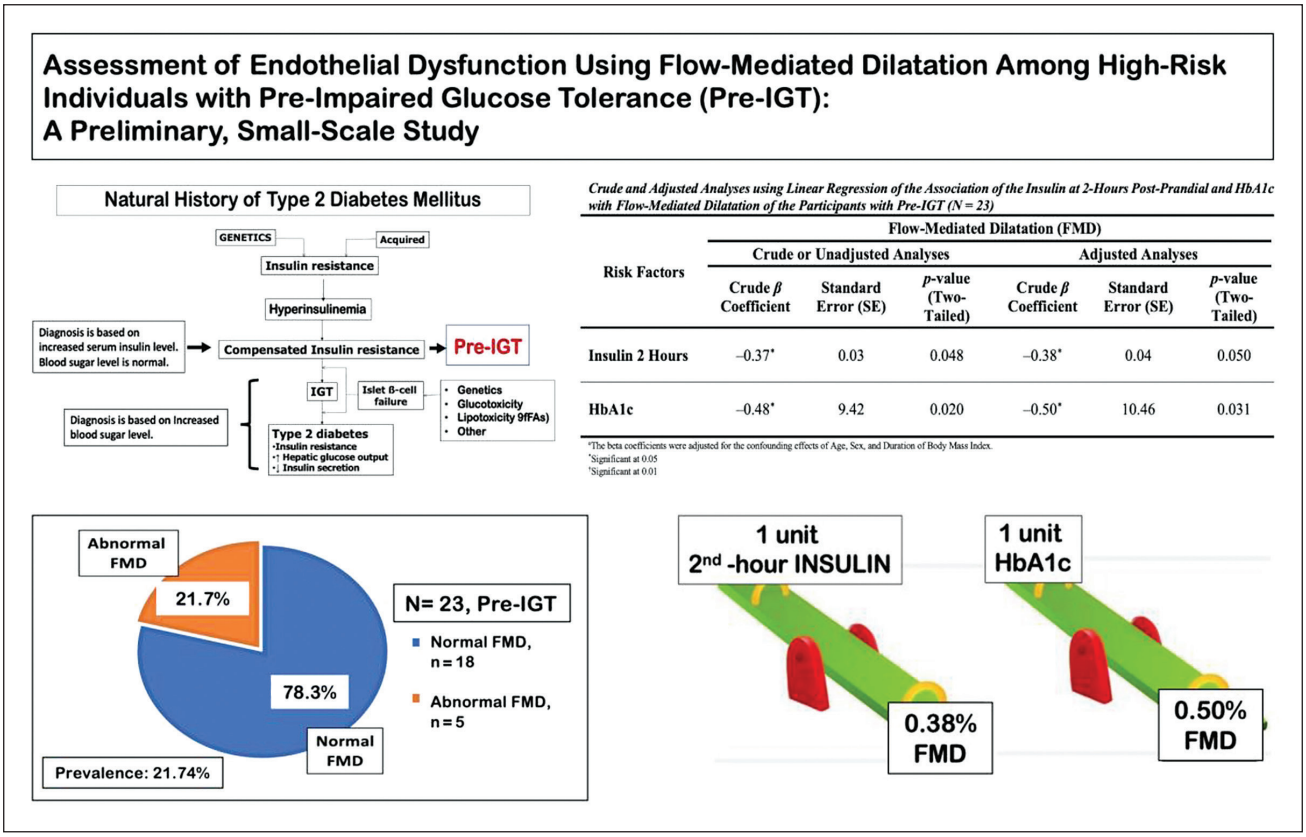

Figure 1. Graphical Abstract.

or screening patients with a hyperinsulinemic pattern, followed by a value >45 uU/mL (sensitivity = 85.00%).[24] Our group[15-17] has been utilizing the 2-hour plasma insulin level >30 mU/mL to identify high-risk individuals with pre-IGT.

Pre-IGT, hyperinsulinemia, and endothelial dysfunction

Pre-IGT as a potential model for CVD outcomes of hyperinsulinemic patients

The influence of various cardiometabolic factors (IGT, T2D, obesity, dyslipidemia and smoking) in ED development makes it challenging to quantify hyperinsulinemia's isolated contribution in assessing CVD development among high-risk individuals with IR. Although the development of vascular functional abnormalities like ED has been extensively elaborated in the prediabetes stage, the abnormal circulating BG levels consistently remained the significant contributory factor of contention in addition to other CVD risk factors like hypertension, dyslipidemia and smoking.[10-13] An example is in the report by Sciacqua et al., where the 1-hour BG post-OGTT of at least 155 mg/dL among the hypertensive participants was the major determinant of all indices of vascular stiffness.[29]

In our present study, we noted that hyperinsulinemia may also be implicated in the development of endothelial dysfunction, and this notion is aligned with the assertions of previous literature in the early identification of populations at risk of DM for prompt diagnosis and clinical management.[30-32] Cabrera de Leon et al., demonstrated in the general population, who were followed up for 3.5 years, an increased risk of myocardial infarction and coronary artery disease by 2.8 times and 2.4 times, respectively, with increased C-peptide levels.[33] Notably, Layton and colleagues have demonstrated that in over 17,000 individuals followed up for 15 to 25 years, those with a genetic predisposition to develop T2D already showed significant differences in the clinical and biochemical markers at an early asymptomatic stage versus those with no genetic risk.[34] Corollary to these reports, albeit statistically insignificant, we noted that patients with asymptomatic ED+ had higher age, BMI and elevated biochemical profiles such as fasting and 2-hour BG, HbA1c, total cholesterol and LDL cholesterol values.

Albeit a preliminary and small-scale study using FMD as a measure of ED and after controlling for significant confounders (age, sex and duration of being overweight or obese), we noted a negative, moderate association between 2-hour serum insulin and HbA1c. This is the first study to report the potential association of ED with hyperinsulinemia in the absence of other major CVD risk factors, and these findings are consistent with the idea that early identification of high-risk individuals with IR may be beneficial for clinical management. This concept has also been advanced in several other studies.[30-32]

Recently, the assessment of endothelial function has become a valuable technique in the study of atherosclerosis.[35] Several studies have shown that the endothelium-dependent

vasomotor function in the brachial and coronary arteries predicts long-term cardiovascular risk. These studies, however, only included a subset of high-risk individuals and the predictive significance in low-risk population is not well documented.[36] Thus, it is imperative to compare ED in the normal population and patients with pre-IGT in the future.

Limitations and recommendations

Albeit the presented findings, this study has certain limitations. The small-scale nature of the study warrants a more significant number of participants for more conclusive, precise results and better generalization. Although our study identified the prevalence of ED and its associations with insulin and HbA1c among high-risk adult patients with no other CVD risk factors, the reported statistics may be overestimated even after sufficient statistical control due to the small sample size. Moreover, the biological plausibility of the presented associations may need to be clarified due to the acquired sample size, further supporting the need for large-scale studies. Certain clinical parameters, such as the 1-hour insulin level post-OGTT, were not measured.

Nonetheless, our findings have shown that ED can potentially occur in the hyperinsulinemic, normoglycemic stage of T2D and thus may support the pre-IGT model in CVD outcome development. This preliminary knowledge provides impetus not only for further research endeavors but also for clinicians to intensify the identification of this subpopulation through sufficient history taking and assessment. Since the implicated contributory factors for such occurrence include CVD risk factors and their duration, thus leading to IR, it is imperative to accentuate the extraction of this information during history taking and assessment. To add to this, the current findings may serve as a stepping stone for future endeavors, especially those with consistent findings, in paving the way for a call to action for robust early diagnosis and management of IR, especially among the members of the population who are not overtly at risk or have no or have unknown CVD risk factors.

CONCLUSION

Pre-IGT is a prediabetes stage characterized by compensatory hyperinsulinemia due to insulin resistance with still normal or absent major cardiometabolic risk factors like hyperglycemia, hypertension and dyslipidemia. In our study, ED was observed in 21.74% of patients. In the absence of other CVD risk factors and at the early stage of T2D, hyperinsulinemia and high HbA1c appear to be associated with endothelial dysfunction.

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

CRediT Author Statement
JAS: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **ALM:** Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – review and editing, Project administration; **RJGS:** Conceptualization, Methodology, Validation, Resources, Data Curation, Writing – review and editing, Project administration; **FP:** Conceptualization, Methodology, Validation, Resources, Data Curation, Writing – review and editing, Project administration; **JRM:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **LMA:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition

Author Disclosure
The authors declared no conflict of interest.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Clinical Profile of Non-thyroidal Cancer Patients with Tyrosine Kinase Inhibitor-induced Thyroid Dysfunction in the University of Santo Tomas Hospital, Philippines: A 5-Year Single-center Retrospective Study

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Abstract

Objectives. This study aimed to determine the clinical profile of non-thyroidal cancer patients with thyroid dysfunction associated with tyrosine kinase inhibitor (TKI) therapy at the University of Santo Tomas Hospital (USTH), Philippines.

Methodology. This is a retrospective observational study of TKI-initiated adult non-thyroidal cancer patients with thyroid function testing from 2013 to 2018.

Results. Forty percent (95% CI: 26.2% - 58.61%) of the sixty individuals who had thyroid function tests (TFT) had incident thyroid dysfunction. Thirty percent had hypothyroidism (i.e., 25% overt [mean TSH 16.64 uIU/mL]; 5% subclinical [mean TSH 6.62 uIU/mL]). The median time at risk was 8 and 16 months for overt and subclinical hypothyroidism, respectively. Fifty-six percent had persistent hypothyroidism (median TSH 16.75, $p = 0.009$). The average time to recovery of transient hypothyroidism was 39 months. Ten percent had hyperthyroidism with a median time at risk of 1.5 months. Non-small cell lung cancer and renal cell carcinoma were possible associated risk factors of thyroid dysfunction.

Conclusion. TKI-induced thyroid dysfunctions are common. Screening and monitoring for thyroid abnormalities during TKI therapy is important.

Key words: tyrosine kinase inhibitors, hypothyroidism, hyperthyroidism

INTRODUCTION

Tyrosine kinase inhibitors (TKI) belong to a class of molecular multi-targeted anti-cancer therapy which target active sites of kinases. This mechanism prevents phosphorylation and subsequently inhibits cell proliferation and angiogenesis.[1] There have been numerous accounts of TKI-induced thyroid dysfunction (i.e., hypothyroidism or hyperthyroidism) with incidence varying from 3.1 to 100% depending on the drug, dose and monitoring protocol.[2]

Hypothyroidism occurred most frequently with sunitinib, one of the earliest TKIs, with a reported incidence of 36-46% in prospective studies. The median time to development of thyroid dysfunction was 4 weeks.[3] In patients treated with sorafenib, the incidence of hypothyroidism is about 18%.[4] Most of the incidence studies focused on a single class of TKI: sunitinib (53-85%), sorafenib (20-36%), imatinib (90-100%) among patients who underwent total thyroidectomy, and axatinib (83-92%).[3] The incidence of hyperthyroidism with sunitinib reached up to 40%.[5] Meanwhile, subclinical and transitory hyperthyroidism has been reported with sunitinib, sorafenib and axatinib.

The possible mechanisms of TKI-induced thyroid dysfunction were studied mostly with sunitinib.[2] Sunitinib likely triggers thyroid dysfunction more frequently because of its broad-spectrum characteristic (i.e., inhibition of VEGF-1, PDGF and VEGF-2) suggesting its important role in the angiogenesis of the thyroid gland.[6] Thyroiditis is believed to be due to TKI-induced cell lysis and devascularization. Other probable mechanisms include reversible reduction of iodine uptake, inhibition of thyroid peroxidase activity and progressive depletion of thyroid functional reserve.[3,4]

The predisposing risk factors for thyroid dysfunction have not been fully determined.[5] According to the study by Lechner and colleagues, patients who developed hypo-

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thyroidism had greater odds of being female as compared to those who remained euthyroid throughout.[7] On the contrary, cumulative TKI exposure duration, greater number of TKI received and the type of cancer do not seem to modify the risk.[2]

In the Philippines, TKIs have been used in various aggressive cancers. Currently, newer classes of TKIs are available which could lead to an increase in TKI-induced thyroid dysfunction. There is no local data on TKI-induced thyroid dysfunction that has been published to date.

Hence, the study aimed to determine the clinical profile of non-thyroidal cancer patients who developed TKI-induced thyroid dysfunction (i.e., thyroiditis-induced thyrotoxicosis, hypothyroidism, worsening of thyroid function/ recurrent hypothyroidism) in USTH from January 2013 to December 2018. This study also aimed to assess the timing of thyroid dysfunction with cumulative TKI exposure.

METHODOLOGY

Study design

This is a retrospective observational study of adult non-thyroidal cancer patients treated with TKI in USTH, Manila, Philippines approved by the Research Ethics Committee.

Study participants

All patients who were on TKIs were screened for eligibility criteria which included the following: (1) non-thyroidal cancer patients >18 years old; (2) initiation of TKI between January 2013 and December 2018 at the USTH; (3) with serial TFT; (4) not on any medications that could alter thyroid function test (e.g., systemic steroids, amiodarone, iodinated contrasts, dopamine, octreotide, metoclopramide). The patients with any of the following were excluded: (a) absent TFT; (b) had previous immune checkpoint inhibitor therapy (ICI); (c) admission for critical conditions and/ or infections (Figure 1).

Sample size

The sample size included all identified eligible patients from the medical oncology residents' database and the individual clinic data of the affiliated oncologists. The total number of patients may be underestimated because no department database included all the patients initiated with TKI. A minimum number of 35 patients was needed to achieve a 95% confidence interval and 10% margin of error based on the highest reported incidence of sunitinib (85%).[2] Sunitinib was used for the sample size computation since it is the most widely studied TKI. Because of the retrospective design of the study and since testing of thyroid function is not routinely done by all specialists, only 60 patients were included in the study. The unavailability of TFT is the most common reason for exclusion from the study.

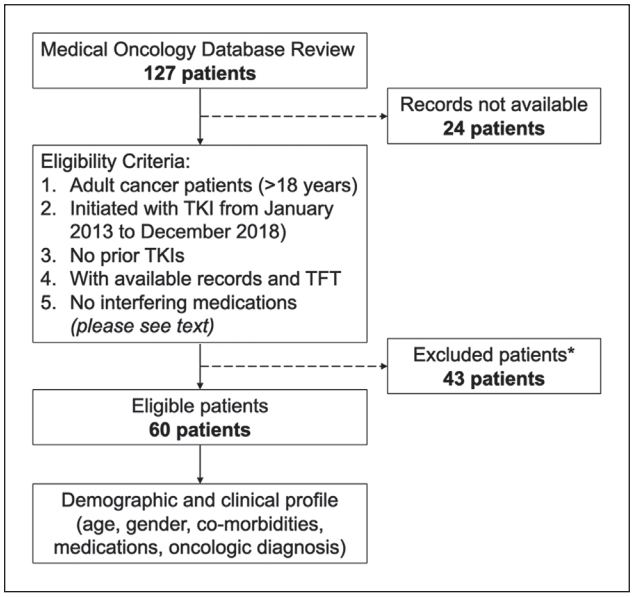

Figure 1. Study Flowchart. Sixty patients satisfied the eligibility criteria.

*Excluded because of lack of TFT monitoring

Study procedure

The patients who received their first dose of TKI treatment between January 2013 to December 2018 with a minimum follow-up period of 2 years after the initiation were included in the study. Data collection included the following: demographic profile, family history of thyroid disorders, cancer diagnosis and stage, comorbidities and the Eastern Cooperative Oncology Group (ECOG) status. Monitoring and treatment of thyroid dysfunction were noted.

Definition of thyroid dysfunction

Thyroid dysfunction was defined based on clinical and biochemical evidence of thyroid disorder. Thyroid Stimulating Hormone (TSH) levels were reported in uIU/mL.
Overt Hyperthyroidism – biochemical evidence of suppressed TSH level, elevated free T4 (FT4) or free T3 (FT3) based on laboratory-specific reference range, clinically hyperthyroid or those requiring anti-thyroid medications
Subclinical hyperthyroidism – asymptomatic patient with suppressed TSH level and normal FT4 or FT3 based on a laboratory-specific reference range
Overt hypothyroidism – biochemical evidence of elevated TSH level, low FT4 based on laboratory-specific reference range, clinically hypothyroid or those requiring thyroid hormone replacement
Subclinical hypothyroidism – TSH 5-10 uIU/mL or higher if FT4 is normal

Study outcomes

The primary outcome is the clinical profile of patients with TKI-induced thyroid dysfunctions. Thyroid dysfunction was stratified according to the class of TKI. The timing of

occurrence of the thyroid dysfunction with cumulative exposure to TKI was also determined.

Statistical analysis

Descriptive statistics was used to summarize the demographic and clinical characteristics of the patients. Mean and SD were used for normally distributed continuous variables, median and interquartile range for non-normally distributed continuous variables and frequency and proportion for categorical variables. Mann-Whitney U test was used to determine the difference between persistent hypothyroid patients versus those non-persistent. Wilcoxon-Signed rank test was used to determine the difference from the baseline to the next time point. One-way ANOVA and Kruskal-Wallis test were used to determine the difference across diagnoses, namely euthyroid, hyperthyroidism, and hypothyroidism. The chi-square test or Fisher's Exact test was used to compare proportions. Crude odds ratios and corresponding 95% confidence intervals from binary logistic regression analysis were computed to assess the possible association of demographic and clinical characteristics with thyroid dysfunction. Null hypotheses were rejected at 0.05 α-level of significance. STATA 13.1 was used for the data analysis.

RESULTS

Of the 127 patients initially identified, only 60 patients satisfied the eligibility criteria. Incident thyroid dysfunction occurred in 40% (95% CI: 26.2% - 58.61%) of the patients. Hypothyroidism occurred in 30% of the patients [25% overt hypothyroidism (mean TSH 16.64 uIU/mL, SD 10.22) and 5% subclinical hypothyroidism (mean TSH 6.21 uIU/mL, SD 3.49)]. Ten percent had hyperthyroidism [3.3% overt hyperthyroidism (mean TSH 0.20 uIU/mL, SD 0.14) and 6.7% subclinical hyperthyroidism (mean TSH 0.20 uIU/mL, SD 0.20)].

The comparison of demographic characteristics among the groups is shown in Table 1. The incidence of thyroid dysfunction differs significantly among cancer types. A greater proportion of patients with non-small cell lung carcinoma (NSCLC) [p = 0.028] and renal cell carcinoma (RCC) [p = 0.008] had overt hypothyroidism. Forty-five percent of patients with diabetes were able to maintain a euthyroid status (p = 0.015). There were no differences in terms of age, gender, ECOG status and cancer stage.

TKI exposure and thyroid dysfunction

The distribution of thyroid dysfunction according to TKI is shown in Figure 2. The majority of the patients received imatinib (21 /60, 35 %). Eighty-two percent of the pazopanib group developed overt hypothyroidism. Aside from pazopanib, overt hypothyroidism occurred in gefitinib (4/12, 33%) and osimertinib (2/2, 100%). Hyperthyroidism developed in both patients treated with bosutinib. Subclinical hypothyroidism occurred in imatinib (1/21, 5%) and afatinib (2/2, 100%) while subclinical hyperthyroidism occurred in imatinib (2/18, 11%) and gefitinib (2/12, 17%). No thyroid dysfunction developed in the sunitinib and sorafenib group. There were no significant differences in thyroid dysfunction between a single TKI and two subsequent TKIs (p = 1.000).

Clinical course of TKI-induced thyroid dysfunction

All patients were biochemically euthyroid (mean TSH 1.25 uIU/mL) at baseline with no significant difference between the initial levels of TFT. The majority remained euthyroid during treatment (59%). Among the hypothyroid group,

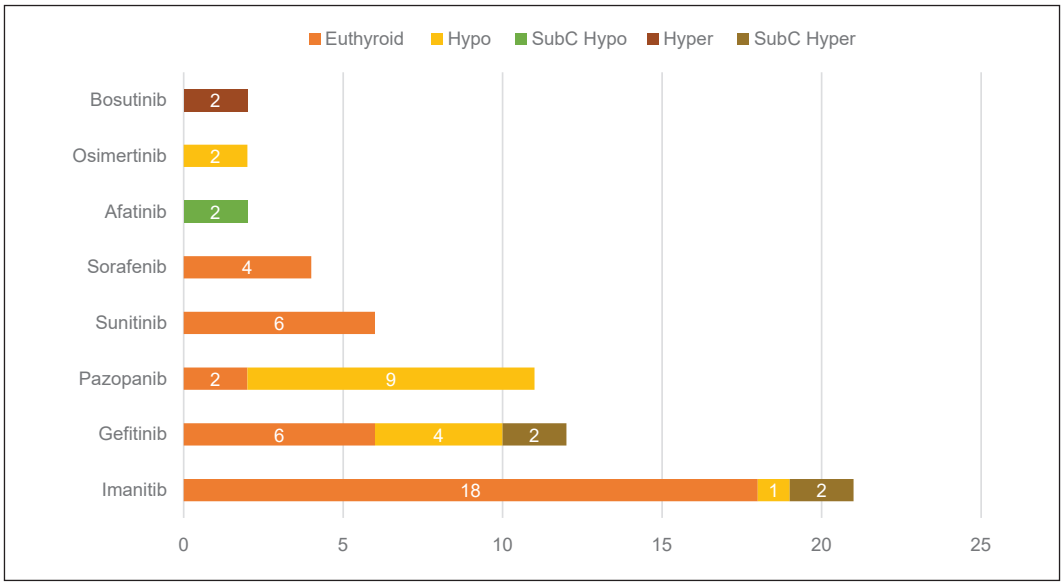

Figure 2. The distribution of patients with thyroid dysfunction* stratified according to TKI (p <0.001).

*Hypo – Overt Hypothyroidism, SubC Hypo – Subclinical hypothyroidism, Hyper – Overt Hyperthyroidism, SubC Hyper – Subclinical hyperthyroidism

the median level of TSH increased to 10.58 uIU/mL on the first follow-up (IQR: 8.57-18.5) from 0.85 uIU/mL (IQR: 0.72-0.98). The median time at risk (i.e., the median time of TKI exposure from initiation to thyroid dysfunction) for overt hypothyroidism was eight months (mean TSH 16.64; IQR: 4.5-12). The median time at risk for subclinical hypothyroidism was 16 months (mean TSH 7.75; IQR: 15.75-27.00). The median TSH level in the hyperthyroid group changed from 1.27 uIU/mL to 0.21 uIU/mL upon the first follow-up. The median time at risk for overt and subclinical hyperthyroidism were 1.5 and 16 months, respectively.

The highest recorded TSH level was 44.01 uIU/mL in an asymptomatic patient two months after pazopanib initiation. This was the shortest time to develop hypothyroidism. The longest time at risk for overt hypothyroidism was 24 months (osimertinib). The maximum median TSH was 14.15 uIU/mL (IQR: 10-19) for overt and 7.75 uIU/mL (IQR: 5.5-10) for subclinical hypothyroidism.

Discontinuation of TKI was made in 93% of overt hypothyroid patients. Sixty-seven percent of the cases were referred to the service of endocrinology. The median TSH of patients referred to endocrinology was 14.65 uIU/mL. Levothyroxine was initiated in 57% of the cases with doses ranging from 50-100 mcg/day.

Persistent hypothyroidism

Seventy-one percent had persistent hypothyroidism (i.e., patients who were maintained on thyroid hormone therapy for at least two years). Table 2 shows the clinical course of patients with persistent hypothyroidism. The median maximum TSH level was significantly higher in the persistent hypothyroid group (16.8 versus 8.9 uIU/mL). All patients with overt hypothyroidism were given thyroid hormone therapy ($p = 0.023$) and were all referred to an endocrinologist ($p = 0.002$). TKIs were resumed after achieving biochemical euthyroidism. In 88% of patients who had transient hypothyroidism, the average time to recovery was 40 months without thyroid hormone replacement.

Table 1. Demographic characteristics of the patients stratified with thyroid dysfunctions

	Diagnosis				*P*-value*
	Total (n = 60)	Euthyroid (n = 36, 60%)	Hyperthyroidism (n = 6, 10%)	Hypothyroidism (n = 18, 30%)	
	Frequency (%); Mean ± SD				
Age	60.58 ± 12.64	59.19 ± 13.7	55.83 ± 16.27	64.94 ± 7.61	0.182
Gender					0.591
Male	30 (50)	16 (44.44)	4 (66.67)	10 (55.56)	
Female	30 (50)	20 (55.56)	2 (33.33)	8 (44.44)	
ECOG					0.060
0	46 (76.67)	30 (83.33)	6 (100)	10 (55.56)	
1	12 (20)	6 (16.67)	0	6 (33.33)	
2	2 (3.33)	0	0	2 (11.11)	
Cancer					
NSCLC	19 (31.67)	7 (19.44)	4 (66.67)	8 (44.44)	0.028
CML	14 (23.33)	12 (33.33)	2 (33.33)	0	0.009
RCC	14 (23.33)	5 (13.89)	0	9 (50)	0.008
Others	13 (21.67)	12 (33.33)	0	1 (5.56)	0.035
Stage (n = 18)					
IIIB	2 (11.11)	2 (16.67)	0	0	1.000
IV	16 (88.89)	10 (83.33)	2 (100)	4 (100)	
Comorbidities					
Hypertension	24 (40)	12 (33.33)	2 (33.33)	10 (55.56)	0.278
Diabetes Mellitus	11 (18.33)	5 (13.89)	4 (66.67)	2 (11.11)	0.015

ECOG – Eastern Cooperative Oncology Group, NSCLC – Non-small cell lung carcinoma, CML – Chronic Myelogenous Leukemia, RCC – Renal Cell Carcinoma
**p*-value less than 0.05 is considered to be statistically significant

Table 2. Clinical course of patients with persistent hypothyroidism

	Total (n = 18)	Persistent (n = 10)	Not persistent (n = 8)	*P*-value*
	Frequency (%); Median (IQR)			
Time to dysfunction (in months)	10 (6 to 16)	10 (2 to 12)	11.5 (7 to 26)	0.264
TSH levels during therapy				
Maximum	11.7 (8.3 to 16.75)	16.75 (12.29 to 22.77)	8.89 (5.61 to 11.45)	0.009
Diagnosis				0.023
Hypothyroidism	14 (77.78)	10 (100)	4 (50)	
Subclinical Hypothyroidism	4 (22.22)	0	4 (50)	
Initial TKI management				1.000
Stopped	16 (88.89)	9 (90)	7 (87.5)	
Maintained	2 (11.11)	1 (10)	1 (12.5)	
Referred to Endocrinologist	12 (66.67)	10 (100)	2 (25)	0.002
LT4 (n=9)				1.000
50-100 ug/day	3 (33.33)	2 (28.57)	1 (50)	
100 ug/day	6 (66.67)	5 (71.43)	1 (50)	
Time to resolution (n=10)	37 (12 to 40)	19 (12 to 26)	39 (24 to 42)	0.237

**p*-value less than 0.05 is considered to be statistically significant

Table 3. Binary logistic regression analysis of the clinical profile of TKI-induced thyroid dysfunction*

Parameters	Crude OR	95% CI	*P*-value
Cancer			
NSCLC	4.1429	1.3123 to 13.078	0.015
CML	0.1818	0.0365 to 0.9049	0.037
RCC	3.7200	1.0604 to 13.050	0.040
TSH on follow-up	1.4806	0.9954 to 2.2024	0.053
Age	1.0234	0.9798 to 1.0689	0.298
Male	1.7500	0.6158 to 4.9728	0.294
ECOG			
0	(reference)	-	-
1	1.8750	0.5192 to 6.7706	0.337
2	-	-	-
Comorbidities			
Hypertension	2.0000	0.6940 to 5.7641	0.199
Diabetes Mellitus	2.0667	0.5514 to 7.7466	0.282

ECOG – Eastern Cooperative Oncology Group, NSCLC – Non-small cell lung carcinoma, CML – Chronic Myelogenous Leukemia, RCC – Renal Cell Carcinoma, OR – Odds Ratio
**p*-value less than 0.05 is considered to be statistically significant

None of the patients who developed hyperthyroidism were given anti-thyroid medications. TKIs were temporarily discontinued in all cases of overt hyperthyroidism. The respective median times to recovery of subclinical and overt hyperthyroidism were 17 and 1.5 months.

Clinical profile of thyroid dysfunction

There was a crude association between cancer and thyroid dysfunction as shown in Table 3. Patients with NSCLC or RCC were more likely to have thyroid dysfunction and CML patients were less likely to have such. No other clinical parameters were significant.

DISCUSSION

Tyrosine kinase inhibitors may induce thyroid dysfunction, more commonly, hypothyroidism. Overall, our study showed an incident thyroid dysfunction (i.e., hyper- and hypothyroidism) of 40% among the patients with thyroid function monitoring. Incident hypothyroidism occurred more frequently. Similar to previous reports, there were only limited reports of TKI-induced thyrotoxicosis and thyroiditis. In a review of the literature by Amahdieh and Salti, isolated hyperthyroidism occurred only in a small percentage of patients given sunitinib (10%), sorafenib (2.6-5%), and axitinib (16%).[3]

Among the TKIs, 47% of hypothyroidism occurred in pazopanib. Pazopanib is a multi-tyrosine kinase inhibitor, targeting VEGFR 1, 2 and 3, c-kit and PDGF receptor.[3] The median TSH level among this group was 16 uIU/mL with the highest recorded at 44 uIU/mL. The median time to occurrence of pazopanib-induced hypothyroidism was six months. Limited reports are available on the occurrence of thyroid dysfunction with pazopanib. In contrast with the present results, hypothyroidism occurred only in less than ten percent of patients in a randomized phase III trial of pazopanib.[8] In a study of 578 patients who received pazopanib from three trials, the incidence of overt hypothyroidism was 6%.[3,9]

Overt hypothyroidism also occurred with osimertinib and gefitinib. Both were not among the TKIs known to develop hypothyroidism in the study by Ahmadieh and Salti.[3] Osimertinib is a third-generation TKI recommended as a third- or later-line treatment for NSCLC.[10] It has a great affinity for mutant EGFR and irreversibly binds various intracellular tyrosine kinase domains.[11] In a multicenter study of advanced NSCLC treated with osimertinib, there were no accounts of thyroid dysfunction.[10,11] Gefitinib inhibits numerous tyrosine kinases including EGFR and is extensively studied in NSCLC. In a prospective observational study, gefitinib was among the TKIs that caused a 4% incident hypothyroidism and subclinical hyperthyroidism in NSCLC patients.[12] Gefitinib, likewise, induced overt hypothyroidism and subclinical hyperthyroidism in this present study.

Thyroid dysfunction was most commonly studied among patients given sunitinib and sorafenib. Most studies on the mechanism and clinical course of TKI-induced hypothyroidism were based on sunitinib. The incidence of sunitinib-induced hypothyroidism ranged between 53 and 85%. Less common than sunitinib, the incidence of thyroid dysfunction in patients on sorafenib ranged between 20 and 36%.[3] This present study showed that none of the patients initiated with both TKIs developed thyroid dysfunction. This may be due to the small number of patients who were on these two medications.

While the majority of patients received imatinib, 82% of these patients maintained euthyroidism throughout. Imatinib was shown to increase the dose of thyroid hormone replacement among post-thyroidectomy patients. In the study by de Groot, all advanced medullary thyroid cancer patients who had previous total thyroidectomy had increased hormone requirement while on imatinib while those who had not undergone thyroidectomy remained euthyroid.[13] None of the patients who received imatinib had previous thyroidectomy or RAI which could explain why no overt hypothyroidism was observed despite the large proportion of patients.

Both patients started on bosutinib therapy developed hyperthyroidism. Bosutinib is a dual Src and ABL1 TKI reserved for resistant CML patients.[14] In one study, 88% of patients on bosutinib developed hypothyroidism and none had hyperthyroidism. Notably, hyperthyroidism preceded hypothyroidism in one of these patients.[15]

The main mechanisms of TKI-induced hypothyroidism include the following: (a) drug-induced thyroid atrophy through inhibition of vascularization; (b) drug-induced thyroiditis; (c) reduction in the synthesis of thyroid hormones; and (d) inhibition of thyroid uptake of iodine.[4,16] The TKI-induced hyperthyroidism is probably secondary to the destructive thyroiditis due to the vascular damage which leads to thyroid atrophy and permanent hypothyroidism which shows the need to monitor patients serially for thyroid dysfunction.[2] This continuum was not observed in

any patients who developed hyperthyroidism in our study. The patients who developed subclinical hyperthyroidism subsequently had normal thyroid function tests after repeat TFT after two weeks. However, controlled serial monitoring of TSH was limited by the retrospective nature of the study.

Overall, the median time at risk for overt hypothyroidism from TKI exposure was eight months (4.5 to 12 months) while for subclinical hypothyroidism was 26 months (16 to 36 months). On the other hand, the median time exposure to the development of overt and subclinical hyperthyroidism was 1.5 and 16 months, respectively. This was comparable to a prospective study by Wolter et.al. in which the median time to abnormal TSH was 1 month (0.5 to 11 months).[17] After 36 months, no further thyroid dysfunctions were observed. Hence, beyond which, TFT monitoring may be decreased. The longest evidence of euthyroidism was observed in an imatinib-treated patient for 182 months. In the study by Wong et al., among patients on sunitinib, elevated TSH occurred after a median exposure of five months.[18] The median level of TSH rose to 10.58 (14.15 for overt) uIU/mL from 0.85 uIU/mL on the first follow-up. On the other hand, the median TSH level in the hyperthyroid group was 0.21 uIU/mL upon the first follow-up, from a baseline level of 1.27 uIU/mL. The increasing TSH levels on follow-up were associated with lower odds of maintaining euthyroidism. This emphasizes the need for serial TFT.

Initial management was temporary discontinuation of TKIs in 93% of overtly hypothyroid patients. Patients with higher TSH levels were referred to endocrinology (median 14.65 uIU/mL). Among these patients, levothyroxine was initiated in 57% with doses ranging from 50-100 mcg/day. Guidelines would recommend starting treatment if the TSH level is above 10 uIU/mL,[17] similar to the major thyroid societies. Some suggest initiating supportive treatment at a TSH level between 5 and 10 uIU/mL if symptomatic.[2] After achieving biochemical euthyroidism with levothyroxine, TKIs may be resumed. In 88% of patients who had transient hypothyroidism, the average time to recovery was 40 months off TKI without thyroid hormone replacement. All of the patients who had transient hypothyroidism only had subclinical dysfunction. In a similar study, serum TSH levels returned to normal within 60 days after permanently discontinuing sunitinib.[16] However, since the majority of the patients would be maintained on TKI due to advanced cancer, levothyroxine therapy and serial TFT monitoring were continued. In comparison with the study of Mannavola et al., 46% of patients on sunitinib had permanent and 25% had transient hypothyroidism. The class of TKIs used and the time exposure at risk did not differ between transient and persistent hypothyroidism. The study showed that the degree of TSH elevation increased in subsequent treatment cycles.[16]

Patients with solid tumors such as non-small cell lung cancer and renal cell carcinoma had a high incidence of hypothyroidism. In the study of Lechner et al., of 538 patients, those who developed hypothyroidism had higher odds of being female. Similarly, a greater number of TKI received was not associated with thyroid dysfunction.[7] In contrast, there were no significant differences found among all the groups and age, ECOG status and cancer stage in our study. The patients who had prior thyroid dysfunction had worsening of their thyroid dysfunction.

In Southeast Asia, a recent study in Thailand was published regarding thyroid dysfunctions among cancer patients who received targeted therapies. Incident thyroid dysfunction with TKI was 14.6% with subclinical hypothyroidism being the most common.[19]

It has been previously recommended to check for TSH levels at each cycle more importantly on the first four cycles[3] and, if with normal results, may be done every three cycles. With our findings, we similarly recommend baseline TSH determination and prior to each treatment cycle. Our present study showed less likelihood of occurrence of thyroid dysfunction beyond 36 months, therefore, monitoring may be decreased thereafter. Observation of patients with subclinical hypothyroidism without thyroid hormone therapy should be done serially as recovery was observed to occur at 40 months. Treatment with levothyroxine should be initiated whenever TSH levels exceed 10 uIU/mL.[3,4,9] Referral to endocrinology should be done among patients with higher TSH levels and in whom levothyroxine replacement should be deemed necessary. Once euthyroidism is achieved with thyroid hormone therapy, TKIs may be resumed and TSH should be serially monitored.

CONCLUSION

Thyroid dysfunction is common among TKI-treated non-thyroidal cancer patients occurring more frequently among NSCLS and RCC. In this study, hypothyroidism is the most common TKI-induced thyroid dysfunction. Serial monitoring of thyroid function is important during TKI therapy.

Limitations

The study has potential limitations. First is the small sample size due to the single-center nature of the study which could affect its applicability in the general population. Analysis for identification of possible associated risk factors for thyroid dysfunction was not made due to the limited sample size. Secondly, thyroid function testing and monitoring were non-uniform and non-standardized owing to the retrospective design of the study. With this study design, the time in months to first follow-up varied among the patients and the time exposures to TKI were based on the timing of repeat TSH determination. We recommend a prospective design to obtain baseline thyroid function testing and controlled serial follow-up determinations in relation to the treatment cycles. A prospective study design could also eliminate possible selection bias brought about by symptom-triggered TSH determinations. Furthermore,

we recommend a larger sample size to confirm the possible association between cancer and thyroid dysfunction and identify other associated risk factors. Lastly, we recommend a multi-center study to increase the generalizability of the study.

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

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

Author Disclosure
The authors declared no conflict of interest.

Data Availability Statement
Datasets are not publicly available because participants in the study did not give written consent for their data to be shared.

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ORIGINAL ARTICLE

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Effect of Maternal Iodine Excess during Pregnancy on Neonatal Thyroid Function and Neurodevelopmental Status at 12 Weeks

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Abstract

Objective. This study aims to determine the effect of iodine excess in pregnant mothers on thyroid function, growth and neurodevelopment in the neonates when assessed at 12 weeks of age.

Methodology. This prospective study enrolled term neonates with birth weight >2500 gm of mothers having urine iodine concentration (UIC) ≥500 µg/L documented in the third trimester of the peripartum period. Neonatal TSH was collected by heel prick on dried blood spots within 24-72 hours of age and measured by time-resolved fluroimmunoassay. Neonates with TSH ≥11 mIU/L at birth were followed up at 2 and 12 weeks to monitor thyroid dysfunction, growth and development.

Results. A total of 2354 (n = 1575 in the delivery room) maternal urine samples were collected of which 598 (25.4%) had elevated UIC. Forty-nine (12.2%) neonates had TSH ≥11mIU/L on newborn screening of whom 18 and 3 neonates had residual elevated TSH at 2 and 12 weeks of life, respectively. Maternal iodine levels correlated weakly with TSH at 2 weeks (*rho* = 0.299; *p* = 0.037). No child required treatment for congenital hypothyroidism. Eight babies additionally had TSH >5 mIU/L at 12 weeks of life. The growth and development of babies with or without TSH elevation was comparable at three months (*p* >0.05).

Conclusion. Maternal iodine excess in pregnancy and peripartum period causes transient hyperthyrotropinemia in neonates that did not affect the growth and development at 3 months of age.

Key words: thyroid, hypothyroidism, iodinization, hyperthyrotropinemia, thyroid function test, urine iodine concentration

INTRODUCTION

Iodine is a trace element that is required for the synthesis of thyroid hormones. Optimal iodine nutritional status during pregnancy is required for normal brain development of progeny during fetal and early postnatal life. The prevalence of iodine deficiency decreased after the successful implantation of a universal salt iodization program.[1,2] However, an emerging concern of thyroiditis with iodine excess is instead now being reported India as well as other countries.[3,4]

Iodine excess is implicated in many thyroid-related disorders such as thyroid nodules, hyperthyroidism and hypothyroidism, thyroid neoplasms, thyroiditis and neonatal hyperthyrotropinemia.[5] Fetuses and neonates are at higher risk of excess iodine exposure due to increased permeability of the skin, increase in iodide trapping process and lower renal iodine clearance. Thus, they are unable to escape from the acute Wolff-Chaikoff effect, which blocks the uptake of iodine by the thyroid gland and impairs thyroid hormone synthesis. This process is further accentuated when povidone-iodine is commonly used as a disinfectant in obstetrics.[6]

There is evidence for the role of maternal iodine deficiency and transient hypothyroidism in shaping neurodevelopmental outcomes in babies.[7,8] However, literature is sparse regarding the effect of maternal iodine excess on neonatal thyroid function.[8,9] How these transient thyroid disturbances affect long-term neurodevelopmental outcomes in children largely remain unexplored at present.

Urine iodine estimation from spot samples has been validated for population screening for iodine excess rather than 24-hour timed samples.[10] The cutoffs vary during pregnancy and lactation and are affected by circadian rhythm in normal individuals.[11]

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We aimed to evaluate the effect of excess maternal iodine during pregnancy on neonatal thyroid function, growth and neurocognitive development in affected babies from birth until 12 weeks.

METHODOLOGY

A prospective observational single-centre study was done in a tertiary care setting, Lok Nayak Hospital, Delhi between November 2017- November 2018. The institutional ethics committee duly approved it. Pregnant women attending antenatal clinics in their third trimester were enrolled in the study. Women without regular antenatal checkups, those with previously detected thyroid dysfunction, and those with a history of intake of drugs which affect thyroid metabolism like lithium, amiodarone, and antepartum hemorrhage were excluded. A gestation assessment was done by first-trimester ultrasonography.

After taking informed consent, demographic details, age and associated comorbidities were recorded on a pro forma. A random urine sample (avoided morning sample) of 20 ml was collected from the pregnant women during the third trimester visit in a wide-mouthed screw-capped plastic bottle and was stored at minus 20°C after immediate transport to the laboratory. The visits in the third trimester were either scheduled visits or during active labor for mothers who chose to deliver in the hospital.

A qualitative iodine estimation was performed using the 'wet digestion method' on urine samples within 48 hours to identify those with iodine concentrations ≥500 μg/L. In this method, urine was digested with chloric acid under mild conditions and iodine concentration was determined manually through its catalytic role in the reduction of ceric ammonium sulfate in the presence of arsenious acid. As the reduction proceeded, the intensity of the colour decreased which was measured in a spectrophotometer at 420 nm. This method used for public health purposes is quick, cost-effective, and reported to give results in close agreement with the gold standard techniques such as neuron-activation analysis or inductively coupled plasma mass spectrometry.[12]

Healthy singleton breastfed term neonates (gestation of completed 37 weeks until 42 weeks) with birth weight >2500 gm who were delivered to mothers with UIC ≥500 μg/L were considered eligible for enrolment. Babies born with congenital malformations, birth asphyxia or who required admission to the neonatal intensive care unit were excluded. Socio-demographic details of the mother like age, parity and iodized salt intake were recorded on a predesigned pro forma.

A heel-prick blood sample was taken from the neonates who fulfilled the eligibility criteria as part of routine newborn screening between 24 to 72 hours of life, preferably at discharge, whichever was later. The sample was spotted on filter paper (Whatman 903-grade paper). A circular dried blood spot (DBS) was punched and subjected to measurement of thyroid stimulating hormone (TSH) using solid phase, two-site fluoroimmunometric assay based on direct sandwich technique (Perkin Elmer Life Sciences, Turku Finland). The manufacturer prepared standards and controls with a haematocrit of 50-55% and the reference standard used was WHO 2nd reference international standards. The limit of detection of the assay was 2 μU/L and the coefficient of variation was less than 5%. The blood sample for confirmation of thyroid hormone levels was collected as a venous sample on follow-up. The values of TSH reported on DBS at birth were in whole blood units and at 2 and 12 weeks were in serum units on venous samples (serum units = 2.2 whole blood units).[13]

Babies who had elevated TSH levels (≥44 mIU/L serum units or ≥20 mIU/L on DBS) on the first screen necessitated treatment for congenital hypothyroidism with oral levothyroxine at 10-15 ug/kg per day and were excluded from the study. For the purpose of this study, babies with TSH ≥11 -44 mIU/L of serum units (≥5 – 20 mIU/L on DBS) were considered for confirmatory testing. The DBS was repunched from a different circle for repeat TSH estimation. Those neonates with elevated TSH values (≥11 mIU/L serum units or ≥5 mIU/L whole blood units) on repunch were followed up to evaluate the thyroid function tests (TFT) on venous blood at 2 weeks of life. Any baby fulfilling the criteria for congenital hypothyroidism on confirmatory testing was started on thyroxine as per protocol.[13]

A venous sample for measurement of TSH, fT3 and fT4 was repeated at 12 weeks of life in those neonates with TSH >5 mIU/L: Venous TSH was estimated using the electrochemiluminescence immunoassay (ECLIA) with a normal range of 0.58 – 5.57 mIU/L (serum units); fT4 and fT3 were measured using equilibrium dialysis or ultrafiltration as a reference method for standardization in the Cobas e 620 autoanalyzer with Elecsys fT3 III/ fT4 II assay. The normal range of fT3 was 3.1-6.8 pmol/L with a measuring range 0.4 - 50 pmol/L. Age appropriate cutoffs were used to interpret fT4 levels as 14.5 – 29 pmol/L on day 0-14 and 13.4 – 44 pmol/L at 12 weeks and a measuring range 0.3 - 100 pmol/L.[14] TSH was considered as raised at levels ≥11 mIU/L (more than 10 mIU/L) after two weeks of age.[13] Transient hyperthyrotropinemia was defined as babies with only TSH elevation and transient hypothyroxinemia was when they had associated low fT4.

Babies were followed up at 12 weeks for measurement of weight and length by the same person to minimize inter-observer bias. Weight was measured on a digital weighing scale with minimal clothing measuring 100 grams. Length was measured using standard procedures on an infantometer with a minimum measure of 0.1 cm. Three consecutive readings were obtained and the average of the 3 readings was considered for all the measurements. Anthropometry was interpreted as per WHO growth charts 2005.[15]

The neurocognitive development of these neonates was assessed using DASII (Development Assessment Scale for Indian Infants) at 12 weeks of completed age by a pediatrician who was part of the study team. Two developmental domains, namely motor and mental domains were separately scored. A score of >70 was considered as normal.[16]

The urine iodine of the mothers of the enrolled neonates was also estimated at 12 weeks postpartum for the persistence of increased urinary iodine excretion. Cut-offs for maternal urine iodine concentration (UIC) were adopted from WHO guidelines.[11] In pregnant mothers, iodine insufficiency was considered at UIC <150 μg/L, above requirements at between 250-499 μg/L and excessive at ≥500 ug/L. A UIC level of <100 ug/L was deemed insufficient and ≥100 ug/L was considered adequate during lactation.[11]

Sample size

Considering a prevalence of 34% obtained from a previous study regarding transient hypothyroidism or persistent hyperthyrotropinemia in neonates born to mothers with excessive iodine intake conducted in Japan by Nishiyama et al.,[9] with 5% absolute precision and confidence interval of 95%, the required sample size was 345. We proposed to take a sample size of 400, considering the attrition to be 10%.

Statistical analysis

STATA 11 software was used for statistical analysis. The Kolmogorov–Smirnov test was used to check the normality of data. Thyroid function tests, anthropometric parameters and developmental quotients were expressed as their means and standard deviations/ median (IQR) as per the normality of data. To compare continuous parameters in two groups for parametric or non-parametric data, Student's t-test or Mann-Whitney U test was used. Spearman correlation coefficient (*rho*) was calculated to check the correlation between two continuous nonparametric variables. The missing values were not adjusted or imputed in the final analysis.

RESULTS

A total of 2610 samples were collected from pregnant mothers out of which 2354 urine samples (1575 in the delivery room and 779 during routine antenatal visits) were processed during the study period. Urine iodine level of ≥500 μg/L was observed in 598 (25.4%) pregnant mothers. After applying the exclusion criteria, 494 pregnant mothers were selected for neonatal evaluation with a total of 400 enrolled after excluding neonates who did not meet the eligibility criteria (Figure 1).

The mean (SD) maternal age was 25.1 (7.3) years and the period of gestation was 38.3 (3.6) weeks. A majority of mothers 207 (52%) were multiparous and 289 (72%) had delivered vaginally. Iodized salt was used by 97.5% of the population.

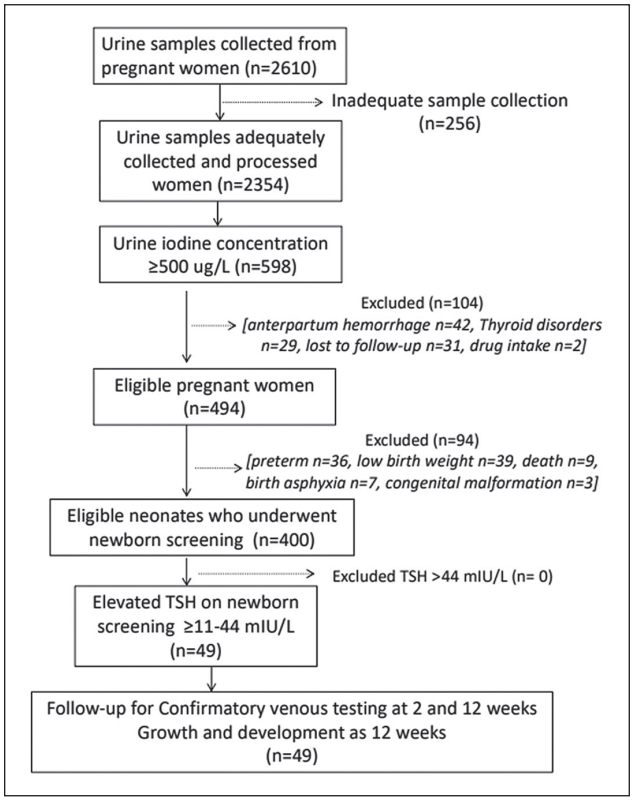

Figure 1. Flow of the study.

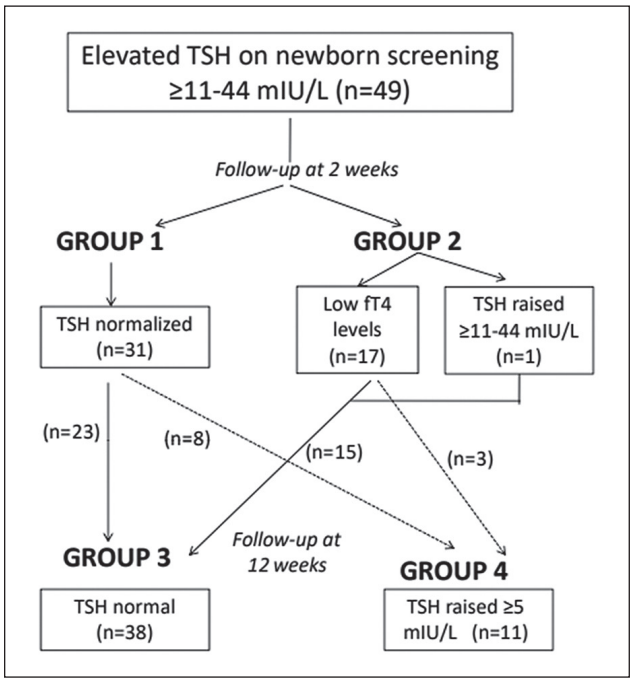

Figure 2. Study groups as per follow-up at 2 and 12 weeks.

The mean (SD) birth weight of neonates enrolled (n = 400; 209 males) was 2872.8 (530.3) grams. A total of 49 (12.2%, 95% CI 15.44%- 18.99%) neonates had TSH ≥11 mIU/L and were followed up till 12 weeks of age, with all of them breastfed. All babies normalized their TSH levels except one baby with TSH of 25.04 mIU/L at day 14 (fT4 14.1 pmol/L) who tested normal on repeat venous sample at day

28 (TSH 15 mIU/L, fT3 4.5 pmol/L, fT4 18 pmol/L) and did not require thyroxine replacement. Eighteen babies (4.5%, 95% CI 2.47% to 6.53%) had low fT4 levels at two weeks. A comparison of neonates who normalized their TSH (Group 1) and those with low fT4 (Group 2) at two weeks is shown in Table 1.

On follow-up, 15/18 babies in Group 2 had normalized the TSH at 12 weeks of age (Group 3). Three babies from Group 2 and eight babies (originally from Group 1) had TSH ≥11 mIU/L (Group 4); Figure 2. Table 2 compares the biochemical parameters in babies who had normalized thyroid functions (Group 3) and those who had a persistent elevation of TSH with normal fT4 levels (Group 4). No baby had hypothyroxinemia at 12 weeks of age.

The median (IQR) maternal UIC at 12 weeks was 84 (40,100) ug/L. The correlation (*rho*) between maternal UIC at 12 weeks and TSH at birth and 12 weeks was weak (*rho* = 0.156; $p = 0.285$) and (*rho* = 0.191; $p = 0.189$), respectively. The maternal UIC correlated significantly, albeit with a weak direct correlation with infant TSH at 2 weeks ($r = 0.299$; $p = 0.037$); (Figure 3). There was no significant correlation between maternal UIC and fT4 at 12 weeks (*rho* = 0.153; $p = 0.295$) or between DASII scores and thyroid hormone levels at any age ($p > 0.05$, data not shown).

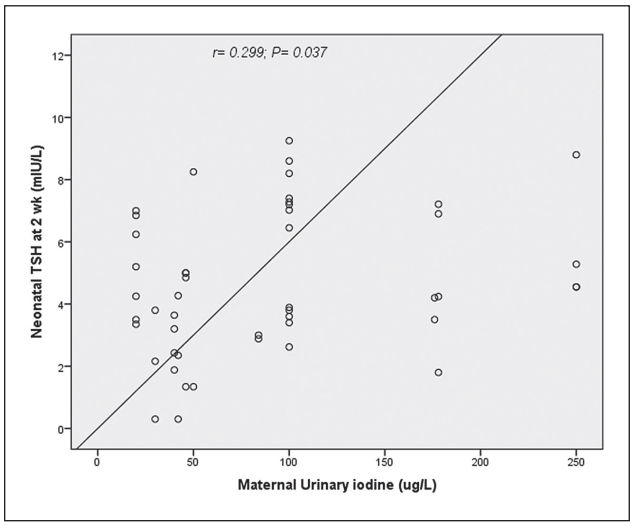

Figure 3. Correlation between maternal urinary iodine concentration and neonatal TSH levels at 2 weeks.

Table 1. Comparison of babies according to thyroid profile at two weeks of life

Parameter	Group 1 (n=31)	Group 2 (n=18)	*P* value
Maternal Age, y	25.4 (3.2)	26.0 (3.7)	0.543
Gestational age, wk	38.6 (1.7)	38.5 (1.2)	0.752
Birth weight, g	2962.4 (538.2)	3017.2 (454.0)	0.718
TSH on DBS[a], mIU/L	7.7 (5.9, 9.8)	9.3 (5.7, 14.9)	0.685
TSH at 2 wk[a], mIU/L	4.6 (3.4, 7.0)	3.8 (2.3, 5.6)	0.175
fT4 at 2 wk, pmol/L	17.8 (2.6)	12.9 (1.8)	<0.001
fT3 at 2 wk, pmol/L	5.1 (1.9)	4.3 (1.3)	0.097
Weight at 12 wk, g	6270.3 (670.2)	5882.3 (659.1)	0.391
Length at 12 wk, cm	60.0 (3.1)	60.0 (2.5)	0.435
TSH at 12 wk[a], mIU/L	3.9 (3.1, 5.2)	3.8 (2.5, 4.9)	0.851
fT4 at 12 wk, pmol/L	16.9 (3.3)	16.2 (2.1)	0.435
fT3 at 12 wk, pmol/L	5.5 (1.6)	4.9 (0.9)	0.211
DASII, mental score	100.9 (7.1)	107.2 (9.1)	0.07
DASII, motor score	100.1 (6.4)	101.9 (6.3)	0.353
Maternal UIC at 12 wk[a], ug/L	100.1 (40.0, 176.0)	46.0 (37.5, 100)	0.247

Data expressed as mean (SD) or [a]median (IQR); Group 1: Babies with raised TSH levels and normal fT4; Group 2: Raised TSH with low fT4; TSH on DBS in whole blood units, TSH on 2 and 12 weeks in serum units; DASII Developmental Assessment Scale for Indian Infants; UIC Urinary iodine concentration at three months post-partum; Comparison by t-test or [a]Mann Whitney U test; P value <0.05 as significant

Table 2. Comparison of babies according to thyroid profile at 12 weeks of life

Parameter	Group 3 (n=38)	Group 4 (n=11)	*P* value
Maternal Age, y	25.6 (3.4)	25.5 (3.5)	0.941
Gestational age, wk	38.4 (1.5)	39.2 (1.8)	0.147
Birth weight, g	2947.1 (512.1)	3105.0 (480.1)	0.366
TSH on DBS[a], mIU/L	7.5 (5.8, 10.2)	9.4 (5.9, 12.7)	0.446
TSH at 2 wk[a], mIU/L	4.1 (3.0, 6.6)	5.3 (3.6,8.8)	0.147
fT4 at 2 wk, pmol/L	15.4 (2.9)	17.8 (3.9)	0.283
fT3 at 2 wk, pmol/L	4.7 (1.4)	5.3 (2.5)	0.032
Weight at 12 wk, g	6112.0 (705.2)	6200.5 (642.3)	0.698
Length at 12 wk, cm	60.0 (2.9)	59.9 (3.0)	0.897
TSH at 12 wk[a], mIU/L	3.5 (2.7, 4.2)	7.2 (6.2, 8.1)	<0.001
fT4 at 12 wk, pmol/L	16.3 (2.2)	17.9 (4.6)	0.276
fT3 at 12 wk, pmol/L	5.1 (1.1)	5.8 (2.1)	0.354
DASII, mental score	102.7 (8.9)	105.0 (6.5)	0.426
DASII, motor score	100.8 (6.9)	100.9 (4.2)	0.937
Maternal UIC at 12 wk[a], ug/L	67.0 (40.0, 100.0)	100.0 (40.0, 250.0)	0.817

Data expressed as mean (SD) or [a]median (IQR); Data compared of the same 49 babies as in table 1; Group 3: Babieswith raised TSH levels and normal fT4; Group 4: Raised TSH with low fT4; TSH on DBS in whole blood units, TSH at 2 and 12 weeks in serum units; DASII Developmental Assessment Scale for Indian Infants; UIC Urinary iodine concentration at three months post-partum; Comparison by t-test or [a]Mann Whitney U test; P value <0.05 as significant

DISCUSSION

This study shows the occurrence of transient thyroid dysfunction in a significant proportion of babies born to mothers with high UIC detected during delivery. However, most of the affected babies recovered by three months of age with normal growth and development in a reassuring manner that reiterates the benign nature of transient high maternal UIC.

Iodine is an essential component of thyroid hormones. Both deficiency and excess of iodine are known to cause adverse effects on human health. Recent studies from India and worldwide have demonstrated an increase in the proportion of goiter and autoimmunity in the post-iodization era. Recent studies from different parts of the world have shown a strong association between iodine excess and thyroid autoimmunity manifesting as goiter, subclinical or overt hypothyroidism.[17,18] Earlier studies have reported up to a 10% incidence of subclinical hypothyroidism in babies with maternal iodine excess,[8] similar to the 12.2% reported in this study. Neonates with maternal iodine excess had a 30% higher TSH in an earlier study.[19] However, the majority of cases of subclinical hypothyroidism were transient in nature without any long-term neurocognitive effects,[8] as seen in this study where transient hypothyroidism recovered by 12 weeks in most babies.

The detection of eight new cases with TSH elevation at 12 weeks of age was a significant finding in this study. Repeat maternal UIC levels were also insufficient suggesting a possible role of environmental goitrogens or iodine deficiency as the likely cause. However, the infant UIC levels, breastmilk iodine levels, maternal thyroid function tests and thyroid antibodies, and consecutive maternal urinary iodine levels that could have suggested the likely etiology were not measured in this study. An earlier study had instead reported higher UIC in infants than their mothers that was predicted by the infant's age and breast milk iodine content.[20] It is also uncertain at present if peripartum maternal iodine excess could have triggered thyroid autoimmunity in these babies, as postulated earlier.[9]

Data from most parts of the world show sufficient median UIC levels among school children and pregnant women after mandatory salt iodization, with few reports of excessive UIC during population screening.[4,21,22] The findings of the present study detected that almost a fourth of the pregnant females with high UIC (>500 ug/L), probably as a result of topical iodine exposure during peripartum period which can result in up to seven times elevation of urinary iodine levels.[23] The maternal UIC levels were insufficient when tested at 12 weeks in all four groups in this study. The estimation of serial urine samples for iodine excretion could have provided information on true iodine excess (not transient) in pregnant mothers. A timed-24-hour sample would have measured UIC with greater precision with lesser individual variations.[24]

The role of thyroid hormones in predicting short and long-term growth in preterm babies has been investigated earlier. The postnatal supplementation of thyroxine for transient hypothyroxinemia was not associated with any beneficial outcomes in preterm babies suggesting it to be a body's adaptive response to sickness or prematurity.[25] The present study also detected 4.5% of babies with hypothyroxinemia (with maternal iodine excess) that was transient in the majority and did not affect the developmental scores. The increased iodine excretion itself could likely have been transient in nature as most mothers normalized the iodine excretion at 12 weeks without a change of dietary habits or residence.

The use of qualitative urinary iodine screening, the lack of serial urinary estimations in the post-partum period, the lack of objective data on dietary iodine consumption and maternal thyroid function status were perceived as limitations of this study. The urinary iodine excretion of newborns was not evaluated as urine collection is cumbersome and difficult at this age. The findings of this study can be assumed to be reassuring for short-term effects, but further studies may be needed to determine the risk of thyroid autoimmunity and long-term effects in the post-iodization era.

To conclude, transient hypothyroxinemia in babies at birth did not impact growth and developmental outcomes at three months of age.

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

CRediT Author Statement
DKR: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Visualization, Project administration, Funding acquisition; **AJ:** Methodology, Software, Validation, Investigation, Resources, Data Curation, Project administration; **AD:** Software, Validation, Formal analysis, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization; **HS:** Methodology, Validation, Formal analysis, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization; **SY:** Conceptualization, Writing – review and editing, Supervision, Project administration; **SK:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Supervision, Project administration, Funding acquisition.

Author Disclosure
The authors declared no conflict of interest.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

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Assessment of Various Insulin Resistance Surrogate Indices in Thai People with Type 2 Diabetes Mellitus

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Abstract

Objective. To compare insulin surrogate indices with the homeostasis model assessment of insulin resistance (HOMA-IR) in Thai people with type 2 diabetes (T2D).

Methodology. A cross-sectional study of 97 individuals with T2D was done to determine the association between HOMA-IR and seven surrogate indices for insulin resistance. IR was defined as HOMA-IR ≥2.0. The indices included Waist Circumference (WC), Waist-to-Hip Ratio (WHR), Waist-to-Height Ratio (WHtR), Triglyceride-Glucose (TyG) index, estimated Glucose Disposal Rate (eGDR) calculated by WC, BMI, and WHR.

Results. A total of 97 subjects with T2D (36.1% female, mean age 61.7 ± 12.0 years, BMI 26.4 ± 3.7 kg/m^2, A1C 6.9 ± 1.2%) were studied. The TyG index showed a positive association with HOMA-IR, while eGDR exhibited a negative association. TyG index had the strongest correlation with IR (r = 0.49), while various eGDR formulas showed weaker negative correlations (r = 0.12-0.25). However, subgroup analysis in individuals with T2D and coronary artery disease (CAD) showed that only eGDR-WC and eGDR-BMI demonstrated a significant correlation with triple vessel disease.

Conclusion. The TyG index was a useful and simple marker for identifying the presence of IR in Thai people with T2D. Future longitudinal studies are warranted to demonstrate the prediction value of cardiovascular outcomes.

Key words: Insulin resistance, Surrogate Markers, HOMA-IR, Triglyceride-Glucose (TyG) index, estimated Glucose Disposal Rate (eGDR)

INTRODUCTION

Insulin resistance (IR) is a major risk factor for developing diabetes complications, especially cardiovascular disease (CVD) among people with type 2 diabetes (T2D).[1] Insulin receptors and their downstream insulin signaling-related molecules play various pathological mechanisms in vascular endothelial cells and macrophages.[2] Changes in insulin signaling activity leads to the onset and progression of atherosclerosis. Although insulin resistance develops more commonly in people with obesity, not all insulin-resistant persons are obese.[3] Other factors leading to insulin resistance could put non-obese people at risk of CVD events. Therefore, several IR surrogate indices have been created in an attempt to quantify the severity of IR in people with and without diabetes.[4-7] The Homeostatic Model Assessment (HOMA-IR) has been widely used in clinical research since 1985 to quantify IR indirectly as the hyperinsulinemic-euglycemic clamp technique is too complex to be used in clinical settings.[4]

However, the HOMA-IR model requires insulin measurement which can be a limitation for low-and middle-income countries (LMICs). Several alternative IR surrogate markers including anthropometry and body composition,[8-10] triglyceride-glucose index (TyG),[11] and estimated Glucose Disposal Rate (eGDR)[12] have subsequently been developed and validated in population-based studies conducted in various parts of the world. The availability of fasting lipid profiles and the known role of hepatic triglyceride content as a strong determinant of insulin resistance in both liver and muscle led to the creation of the TyG index in 2008 by using fasting plasma glucose (FPG) and triglyceride (TG) levels to provide an estimate of IR.[11] Later studies also found that the TyG index could be an independent predictor of unfavorable cardiovascular outcomes in people with T2D.[13-15] On the other hand, eGDR which was proposed earlier in 2000 by using the available clinical factors such as waist circumference (WC), presence or absence of hypertension, and glycated hemoglobin (A1C) was developed to estimate IR in people with type

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1 diabetes (T1D).[12] The utility of eGDR as a measure of IR was validated in more diverse populations, including predicting survival in people with T2D.[16] The use of these instruments for identifying high-risk individuals with IR could assist clinicians in prioritizing interventions in resource-constrained settings. Moreover, the IR-associated co-morbidities could also be targeted to prevent or delay the progression to advanced stages in people with IR.

Unfortunately, to date, there have been few cohort studies conducted in the Southeast Asian population to assess various insulin resistance surrogate indices among the general population. Furthermore, there has been no dedicated study among the Southeast Asian population to evaluate various insulin resistance surrogate markers in individuals with type 2 diabetes, with or without atherosclerotic cardiovascular disease (ASCVD). Moreover, the strength of these surrogate markers could be different according to the ethnicity of the study populations. In the present study, we aim to evaluate various simple insulin surrogate indices with the HOMA-IR in Thai people with T2D and compare the performance of TyG and eGDR in predicting the severity of coronary artery disease (CAD) among T2D with CAD.

METHODOLOGY

This cross-sectional study included Thai adults with T2D who had regular follow-up visits at Theptarin Hospital, a tertiary center in diabetes care in Bangkok, Thailand, between January and June 2023. Participant inclusion criteria included (1) diagnosis of T2D and (2) completed surveillance of diabetes complications. All eligible patients were sequentially invited to participate in the study through consecutive non-random sampling. Exclusion criteria included (1) age <15 years old; (2) participants who are unable to accurately obtain anthropometric measurements; (3) active malignancy or malignant diseases within 1 year of completed treatment (4) changes in weight ≥5% within 6 months before enrollment (5) fasting plasma insulin <2 mU/L or >100 mU/L. This study was approved by the Institutional Review Board Committee of Theptarin Hospital (EC No.02-2022). The study was registered with the clinical trial registry on 04/08/2022, with identifier number TCTR20220804006. Before participating in the study, all participants provided written informed consent.

Sample size calculation

The prevalence of insulin resistance among Thai adults was 25.1%.[17] According to the study by Guerrero-Romero et al., the TyG index showed sensitivity and specificity rates of 96.5% and 85.0% for diagnosing insulin resistance, respectively.[18] Using the Buderer Formula,[19] minimum sample sizes of 52 and 66 were calculated, assuming α of 0.05, β of 0.80, and a 95% confidence interval.

For eGDR-WC, sensitivity and specificity were reported at 83.3% and 79.8% respectively,[12] resulting in minimal sample sizes of 214 for sensitivity and 83 for specificity. Regarding eGDR-WHR, specificity was 83.3% and sensitivity was 86.7%,[12] leading to minimal sample sizes of 72 and 178. To the best of our knowledge, the eGDR-BMI has recently been proposed to be associated with insulin resistance; however, its sensitivity and specificity have not yet been demonstrated. In accordance with this sample size calculation, the recommended sample size was 214 participants. Due to budgetary and time constraints imposed by the grant and the associated laboratory costs, we were only able to enroll the maximum number of cases feasible within these limitations.

Data collection and definitions

Participants underwent routine clinical physical examination, which included the collection of overnight fasting venous blood samples and measurement of weight, height, waist circumference, and resting blood pressure. Weight was determined without shoes by using an automatic electronic scale (Tanita Corp., Tokyo, Japan) to the nearest 100 grams. Standing height was determined without shoes by a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference (WC) was measured in the horizontal plane midway between the lowest ribs and the iliac crest. Hip circumference was measured across the broadest part of the buttocks. Waist-related anthropometric measures including WC, waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) as predictors of IR were studied. The data on patient characteristics, smoking status, glycemic and lipid management, insulin usage, diabetic complications, and co-morbidities were collected. In patients with established ASCVD, significant CAD was defined as more than 50% angiographic diameter stenosis in one or more of the epicardial coronary arteries. Triple-vessel disease was defined as the involvement of any three or more arteries.

The prevalence of IR was estimated by the HOMA-IR method which was calculated with the formula: fasting plasma glucose (mmol/L) times fasting serum insulin (mU/L) divided by 22.5. Based on a previous study in the Asian population, insulin resistance was defined by a HOMA-IR index ≥2.0, which is the value that predicts the development of diabetes more accurately and correlates with the hyperglycemic–hyperinsulinemic clamp method.[20] Participants with a HOMA-IR ≥2.0 were categorized into the insulin-resistant group, and patients with a HOMA-IR <2.0 were categorized into the insulin-sensitive group.

Clinical laboratory analyses

Fasting plasma glucose concentrations (FPG) were determined using the hexokinase method. Fasting plasma insulin concentrations were measured using a solid-phase, two-site chemiluminescent immunometric assay (Immulite 1000, Insulin) with an inter-assay coefficient of variation at 3.3%. Plasma TG concentrations were determined using

standardized enzymatic glycerol phosphate oxidase assay procedures.

TyG index was calculated according to the following equation: Ln[FPG(mg/dl) × TG (mg/dl)/2].[11] eGDR was calculated according to the following formula: eGDR-WC = 21.16 - (0.09 x WC) - (3.41 x hypertension) - (0.55 x A1C) or eGDR-WHR = 24.31 - (12.22 x WHR) - (3.29 x hypertension) - (0.57 x A1C) or eGDR-BMI = 19.02 - (0.22 x BMI) - (3.26 x hypertension) - (0.61 x A1C) [hypertension (yes = 1/no = 0), A1C = A1C in %)].[12]

Statistical analysis

Descriptive statistics for the categorical variables were assessed using the $\chi 2$ test or Fisher's exact test as appropriate, and for the continuous variables, either an independent t-test or Wilcoxon signed-ranks test was employed when applicable. The Shapiro-Wilk test was used to assess normality. Data for continuous variables with skewed distribution was expressed as median (interquartile range). Various IR surrogate markers were stratified into quartiles and logistic regression analysis was used to determine the association between various surrogate markers with insulin resistance status. The associations between each IR surrogate marker and the presence of insulin resistance status were determined using Spearman's rank correlation coefficients or Pearson's correlation, depending on the type of relationship. Based on a previous study addressing confounders[21] and general knowledge, we created 3 models: model 1 was unadjusted, model 2 included adjustment for age and sex, and model 3 was adjusted for age, sex, smoking status, the duration of diabetes and the use of metformin, insulin, thiazolidinedione, and statins for the multivariate model. Finally, we performed subgroup analysis in participants with CAD to evaluate the association between the TyG index and eGDR formulas in identifying participants with multi-vessel disease. A *p*-value of <0.05 was considered statistically significant. All analyses were conducted using the SPSS Statistical Package, version 20 (IBM Corp., Armonk, NY, USA).

RESULTS

Baseline characteristics of the patients

A total of 97 Thai adults with T2D (36.1% female, mean age 61.7 ± 12.0 years, median duration of diabetes 16 years, BMI 26.4 ± 3.7 kg/m^2, A1C 6.9 ± 1.2% were enrolled as shown in Figure 1. The mean HOMA-IR in all participants was 3.8 ± 3.0 and the prevalence of IR estimated by HOMA-IR method was 71.1%. Participants with IR (mean HOMA-IR at 4.7) showed younger age and were more obese than those with no IR (mean HOMA-IR at 1.4) as revealed in Table 1. Regarding waist-related anthropometric measures, WC and WHtR were found to be statistically significantly higher than those with no IR, while WHR was not. The mean value of the TyG index also showed statistically significant differences between groups. However, only eGDR calculated by WC and eGDR calculated by WHR showed lower values in participants with IR, whereas eGDR calculated by BMI did not.

Relationship between various IR surrogate markers for identifying IR

All waist-related anthropometric measures and TyG index were positively associated with the HOMA-IR but various eGDR formulas were negatively associated with the HOMA-IR. Based on correlation analysis, the TyG index yielded the most correlation with the presence of IR (moderately positive correlation at r = 0.49). eGDR calculated by WC, WHR, and BMI showed poor correlation with the HOMA-IR (r = 0.25, 0.12, and 0.23 respectively), as shown in the correlation heatmap in Figure 2.

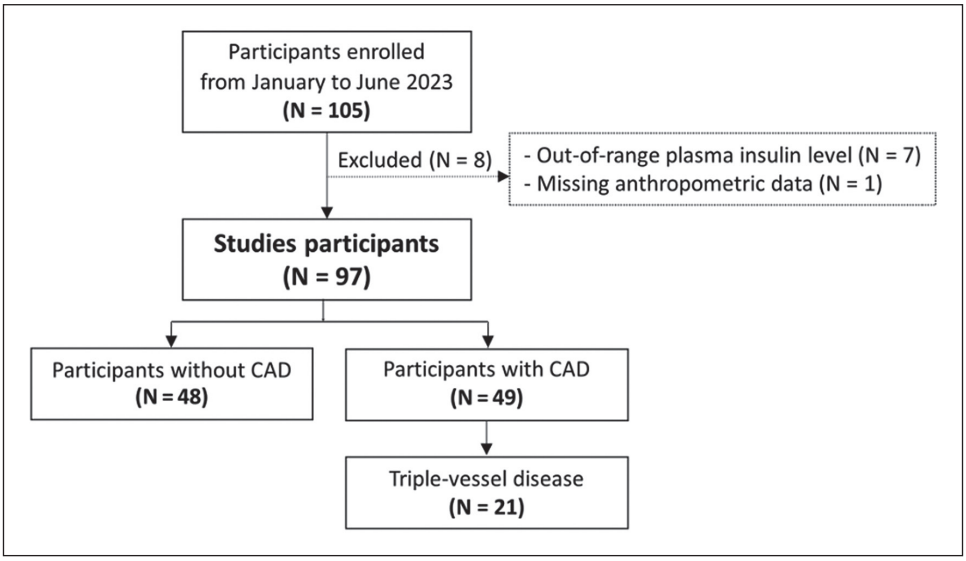

Figure 1. Flow diagram of studied patients (N=97).

The odds ratio for the presence of IR according to each quartile of the TyG index and eGDR formula

Table 2 shows the results of logistic regression of the TyG index and eGDR formula in which model 1 shows unadjusted values whereas models 2 and 3 show values derived after adjusting for potential confounders for the multivariate model. The highest quartile of the TyG index (>9.22) showed an odds ratio for the presence of IR in all models of more than 10 times higher when compared with the lowest quartile of the TyG index (<8.47). Only the lowest quartile of eGDR calculated by WC (<5.37) was statistically significant in all models when compared with the highest quartile of eGDR calculated by WC (>8.73).

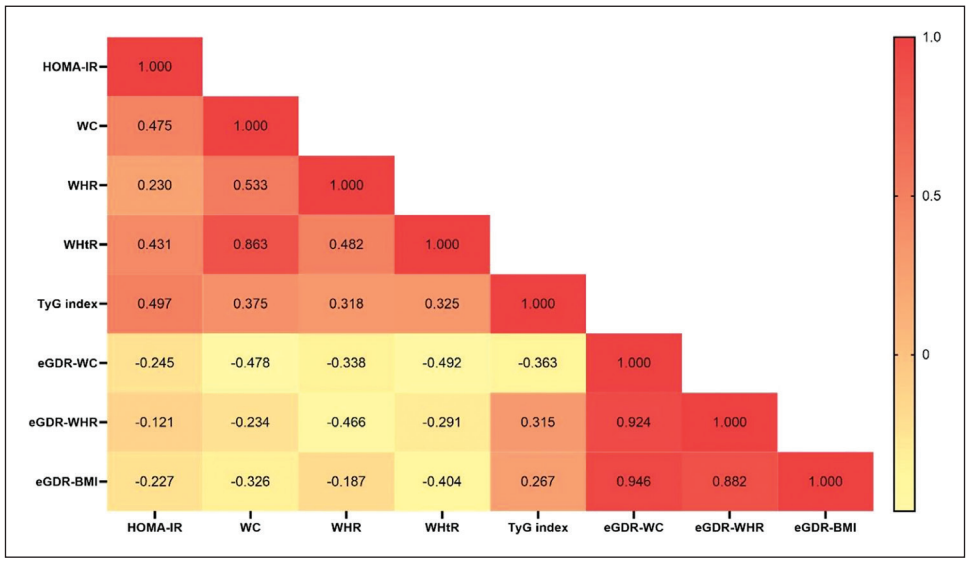

Figure 2. Correlations between HOMA-IR and various insulin indices.

Table 1. Clinical characteristics and laboratory data of studied participants (N = 97)

	Total participants (N = 97)	Participants with HOMA-IR <2.0 (N = 28)	Participants with HOMA-IR ≥2.0 (N = 69)	*P*-value
Age (yrs)	61.7 ± 12.0	64.2 ± 11.3	60.7±12.2	0.193[a]
Female (%)	36.1	42.9	33.3	0.376[b]
Duration of DM (yrs)	16.0 (5.5,25.0)	16.5 (13.2,33.0)	15.0 (5.0,23.0)	0.624[c]
BMI (kg/m²)	26.3 (23.4,28.7)	24.0 (22.7,27.6)	26.4 (24.1,29.6)	0.007[c]
Waist circumference (WC) (cm)	94.0 (86.5-99.5)	90.0 (84.0,94.0)	95.0 (89.0,102.0)	0.002[c]
Hip circumference (HC) (cm)	98.0 (92.5-105.0)	96.0 (90.0,99.0)	99.0 (95.5,105.5)	0.008[c]
Waist-to-hip ratio (WHR)	0.95 ± 0.06	0.94 ± 0.05	0.96 ± 0.06	0.126[a]
Waist-to-height ratio (WHtR)	0.56 (0.52,0.61)	0.54 (0.51,0.58)	0.57 (0.54,0.62)	0.024[c]
Smoking (%)	16.5	21.4	14.5	0.404[d]
Presence of hypertension (%)	63.9	64.3	63.8	0.962[b]
Diabetic retinopathy (%)	28.9	35.7	26.1	0.343[b]
Diabetic kidney disease (%)	27.8	39.3	23.2	0.109[b]
Diabetic neuropathy (%)	20.6	32.1	15.9	0.074[b]
Coronary artery disease (%)	50.5	60.7	46.4	0.201[b]
Triple-vessel disease (%)	42.9	41.2	43.8	0.862[b]
Insulin usage (%)	24.7	21.4	26.1	0.630[b]
Fasting plasma glucose (mg/dL)	124 (109,146)	112.5 (105.8,139.5)	126 (112,148)	0.052[c]
Total cholesterol (mg/dL)	148 ± 29	149 ± 38	148 ± 24	0.851[a]
Fasting plasma triglyceride (mg/dL)	112 (83,148)	88.5 (64.8,111.5)	122 (91,169)	<0.001[c]
Plasma HDL (mg/dL)	56 ± 13	60 ± 11	55 ± 14	0.086[a]
Plasma LDL (mg/dL)	76 (63,91)	77 (58,90)	76 (64,96)	0.720[c]
A1C (%)	6.8 (6.2-7.5)	6.5 (5.5,7.1)	6.9 (6.4,7.6)	0.029[c]
Fasting plasma insulin (mg/dL)	9.4 (5.6-15.5)	4.9 (3.6,5.7)	11.8 (8.9-18.6)	<0.001[c]
HOMA-IR	2.7 (1.8-4.8)	1.5 (1.1,1.8)	3.7 (2.5,6.0)	<0.001[c]
Triglyceride-glucose index	8.9 ± 0.5	8.6 ± 0.4	9.0 ± 0.5	<0.001[a]
Estimated glucose disposal rate (eGDR) calculated by WC	6.6 (5.4,8.7)	7.1 (6.4,8.8)	6.2 (5.0,8.7)	0.019[c]
Estimated glucose disposal rate (eGDR) calculated by WHR	6.2 (5.1,8.6)	6.4 (5.8,8.8)	6.0 (4.8,8.6)	0.168[c]
Estimated glucose disposal rate (eGDR) calculated by BMI	6.4 (5.4,8.7)	7.2 (6.4,8.3)	5.9 (5.2,9.1)	0.021[c]

[a] Independent t-test
[b] Chi-square test
[c] Wilcoxon signed-rank test
[d] Fisher's exact test
Continuous data were presented as means ± SD or median (IQR); categorical data were presented as number (%)

Table 2. Multivariate logistic regression of different indices for predicting the presence of insulin resistance (Model 1: unadjusted; Model 2: adjusted for age and sex; Model 3: adjusted for age, sex, duration of diabetes, smoking, the usage of insulin, metformin, thiazolidinedione and statin for the multivariate model)

Parameter	Model 1, OR (95%CI)	*p*-value	Model 2, OR (95%CI)	*p*-value	Model 3, OR (95%CI)	*p*-value
WC						
Q1; <86.5	Reference		Reference		Reference	
Q2; 86.5-93.9	2.00 (0.62-6.42)	0.244	1.84 (0.56-6.09)	0.315	1.05 (0.25-4.53)	0.944
Q3; 94.0-99.5	4.00 (1.13-14.18)	**0.032**	4.67 (1.26-17.25)	**0.021**	6.68 (1.23-36.41)	**0.028**
Q4; >99.5	7.00 (1.64-29.85)	**0.009**	6.81 (1.57-29.60)	**0.011**	5.76 (1.20-27.64)	**0.029**
WHR						
Q1; <0.91	Reference		Reference		Reference	
Q2; 0.91-0.95	2.40 (0.67-8.65)	0.181	1.99 (0.52-7.43)	0.306	1.73 (0.42-7.22)	0.451
Q3; 0.96-0.98	1.00 (0.31-3.22)	1.000	0.83 (0.24-2.89)	0.772	0.71 (0.18-2.76)	0.617
Q4; >0.98	2.28 (0.63-8.25)	0.209	2.18 (0.59-8.07)	0.243	1.92 (0.48-7.72)	0.357
WHtR						
Q1; <0.52	Reference		Reference		Reference	
Q2; 0.52-0.56	1.73 (0.54-5.53)	0.355	2.49 (0.71-8.76)	0.156	2.87 (0.69-11.8)	0.145
Q3; 0.57-0.61	6.77 (1.61-28.54)	**0.009**	11.37 (2.35-54.9)	**0.002**	9.98 (1.84-54.17)	**0.008**
Q4; >0.61	3.51 (0.99-12.35)	0.051	6.85 (1.57-29.9)	**0.011**	5.40 (1.14-25.58)	**0.033**
TyG index						
Q1; <8.47	Reference		Reference		Reference	
Q2; 8.47-8.90	2.13 (0.67-6.78)	0.203	2.74 (0.80-9.53)	0.108	5.84 (1.26-27.14)	**0.024**
Q3; 8.91-9.22	3.00 (0.88-10.18)	0.078	3.42 (0.97-12.05)	0.056	5.87 (1.29-26.62)	**0.022**
Q4; >9.22	11.00 (2.10-57.50)	**0.004**	11.7 (2.19-62.26)	**0.004**	19.80 (2.82-139.13)	**0.003**
eGDR-WC						
Q1; <5.37	9.47 (1.06-84.37)	**0.044**	15.11 (1.58-144.66)	**0.018**	31.68 (1.95-513.54)	**0.015**
Q2; 5.37-6.62	1.06 (0.31-3.66)	0.928	1.82 (0.45-7.28)	0.399	1.92 (0.39-9.50)	0.423
Q3; 6.63-8.73	0.35 (0.11-1.15)	0.083	0.40 (0.12-1.40)	0.152	0.37 (0.07-1.75)	0.209
Q4; >8.73	Reference		Reference		Reference	
eGDR-WHR						
Q1; <5.14	2.88 (0.65-12.87)	0.165	4.74 (0.94-23.94)	0.059	7.55 (1.11-51.27)	**0.039**
Q2; 5.14-6.22	0.73 (0.22-2.43)	0.611	1.07 (0.29-3.91)	0.916	1.05 (0.24-4.64)	0.947
Q3; 6.23-8.61	0.69 (0.21-2.29)	0.541	0.85 (0.24-2.99)	0.803	0.92 (0.23-3.62)	0.902
Q4; >8.61	Reference		Reference		Reference	
eGDR-BMI						
Q1; <5.37	6.05 (0.65-56.37)	0.114	7.58 (0.78-73.82)	0.081	13.02 (0.71-238.92)	0.084
Q2; 5.37-6.44	0.68 (0.19-2.52)	0.561	0.96 (0.23-4.08)	0.961	0.62 (0.11-3.49)	0.583
Q3; 6.45-8.70	0.16 (0.04-0.57)	**0.005**	0.20 (0.05-0.75)	**0.017**	0.11 (0.02-0.58)	**0.009**
Q4; >8.70	Reference		Reference		Reference	

OR = Odds Ratio, CI = Confidence Interval

ROC analysis using the TyG index and eGDR calculated by WC for identifying IR

The results of ROC analysis using the TyG index and eGDR calculated by WC for identifying IR are shown in Figure 3. The optimal cut-off values, using Youden's index for the TyG index and eGDR calculated by WC were 9.04 (sensitivity 50.7%, specificity 60.5%) and 6.59 (sensitivity 59.4%, specificity 75.0%), respectively.

Performance of TyG and eGDR formulas in predicting the severity of CAD among T2D with CAD

A total of 49 T2D with CAD (20.4% female, mean age 67.5±9.4 years, median duration of diabetes 23 years, BMI 25.8±4.1 kg/m^2, A1C 7.1±1.4%) were analyzed in the subgroup of this cohort. Triple-vessel disease (TVD) was found in 42.8% of these participants as revealed in Table 3. Among T2D with CAD group, the TyG index was found to have no significant correlation with the presence of triple-vessel disease (r = 0.08, p = 0.57). Only eGDR calculated by WC and BMI showed a significant moderate correlation with triple-vessel disease (r = -0.34, -0.33 respectively).

DISCUSSION

In the present cross-sectional study, we confirmed that the TyG index was the reliable surrogate marker for IR among Thai people with T2D. Measures of plasma lipid concentrations are readily available in routine clinical practice and standardized to a much greater degree than assays of fasting plasma insulin concentration. Additionally, besides being a marker associated with IR, the TyG index is also a valid marker for risk stratification of participants with T2D.[22-24] Although measures of IR have not yet been integrated into clinical guidelines, several studies have confirmed the clinical significance of IR beyond glycemic control alone in people with T2D.[1-3] Therefore, the presence of IR should also be considered as one of the targets for improving diabetes management.

Obesity alone does not adequately reflect the different obesity phenotypes as the distribution of adiposity is also important.[3] There is an accumulating body of evidence that gluteofemoral adipose tissue may even be protective.[25] In our study, waist-related anthropometric measures correlated positively with the HOMA-IR but their predictive

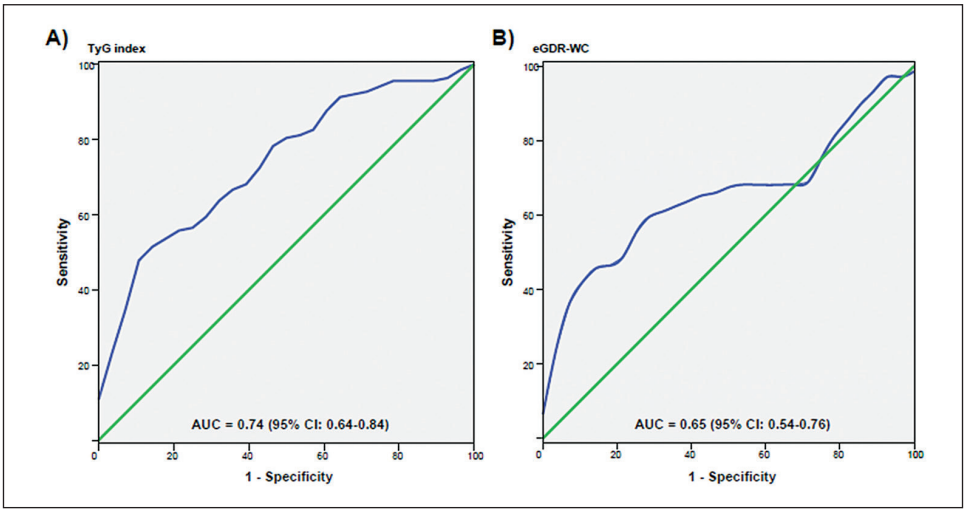

Figure 3. Receiver operating characteristic analysis for predicting the presence of insulin resistance defined by the HOMA-IR from **(A)** TyG index and **(B)** eGDR calculated by waist circumference.

Table 3. Clinical characteristics and laboratory data of studied participants with CAD (N = 49)

	Total participants (N =49)	Participants with triple-vessel disease (N = 21)	Participants without triple-vessel disease (N = 28)	*P*-value
Age (yrs)	67.5±9.4	67.5±10.0	67.6±9.0	0.972[a]
Female (%)	20.4	19.0	21.4	0.565[d]
Duration of DM (yrs)	22.9±12.2	23.9±11.0	22.2±13.2	0.638[a]
BMI (kg/m²)	24.4 (22.9,28.3)	24.0 (22.8,29.8)	24.7 (23.3,26.7)	0.888[c]
Waist circumference (WC) (cm)	93.0 (86.0,99.5)	95.0 (86.0,110.0)	92.0 (86.0,98.8)	0.384[c]
Hip circumference (HC) (cm)	96.0 (90.5,104.0)	97.0,90.5,109.0	96.0 (90.3,100.1)	0.110[c]
Waist-to-hip ratio (WHR)	0.96±0.07	0.96±0.07	0.97±0.07	0.622[a]
Waist-to-height ratio (WHtR)	0.56 (0.52,0.62)	0.55 (0.52,0.63)	0.56 (0.52,0.59)	0.747[c]
Smoking (%)	20.4	23.8	17.9	0.609[d]
Presence of hypertension (%)	83.7	95.2	75.0	0.062[d]
Diabetic retinopathy (%)	44.9	47.6	42.9	0.740[b]
Diabetic kidney disease (%)	53.1	42.9	60.7	0.215[b]
Diabetic neuropathy (%)	34.7	42.9	28.6	0.299[b]
Insulin usage (%)	38.8	47.6	32.1	0.271[b]
Fasting plasma glucose (mg/dL)	125 (109,155)	143 (106,170)	124 (110,140)	0.284[c]
Total cholesterol (mg/dL)	135 (118,153)	132 (114,154)	135 (119,151)	0.801[c]
Fasting plasma triglyceride (mg/dL)	114 (73,149)	111 (77,138)	115 (65,150)	0.816[c]
Plasma HDL (mg/dL)	54±12	54±13	54±12	0.982[a]
Plasma LDL (mg/dL)	67 (52,83)	65 (43,81)	68 (54,84)	0.396[c]
A1C (%)	6.9 (6.0,7.7)	7.2 (6.0,8.0)	6.8 (6.0,7.5)	0.327[c]
Fasting plasma insulin (mg/dL)	7.9 (5.2,13.8)	7.0 (4.2,18.0)	8.0 (5.5,11.3)	0.856[c]
HOMA-IR	2.3 (1.7,4.4)	2.1 (1.5,6.6)	2.3 (1.8,3.7)	0.944[c]
Triglyceride-glucose index	8.9±0.5	8.9±0.6	8.8±0.5	0.570[a]
Estimated glucose disposal rate (eGDR) calculated by WC	6.1±1.9	5.4±1.5	6.7±1.9	0.017[a]
Estimated glucose disposal rate (eGDR) calculated by WHR	6.3±1.8	5.3±1.3	6.1±2.1	0.127[a]
Estimated glucose disposal rate (eGDR) calculated by BMI	5.8±1.8	5.6±1.5	6.8±1.8	0.020[a]

[a] Independent t-test
[b] Chi-square test
[c] Wilcoxon signed-rank test
[d] Fisher's exact test
Continuous data were presented as means ± SD or median (IQR); categorical data were presented as number (%)

values were inferior to the TyG index. IR is an important risk factor for atherosclerosis and a predictor of adverse cardiovascular events after revascularization in patients with CAD.[26] People with diabetes are more likely to have diffuse and multivessel vascular lesions and represent a challenging group of the population of candidates eligible for revascularization techniques.[27] Previous studies demonstrated the role of both the TyG index and eGDR as an indicator of severe CAD in the general population.[28-30] Several possible explanations for these findings present TG and TG-rich lipoprotein (TGRL) as the main causes of residual ASCVD despite statin use.[31] Elevated plasma TG serves as a marker for TGRL and their remnants which up-regulate inflammation, oxidative stress, and foam cell formation in vascular endothelial cells and macrophages.[32] Therefore, elevated plasma TG is associated with the activating process

of atherosclerosis even in patients with low LDL-C levels. Our present study showed that only eGDR-WC and eGDR-BMI demonstrated a significant correlation with triple vessel disease among subgroup analysis in individuals with T2D and CAD. It might be explained by the parameters of glycemic status (A1C results) and the presence of hypertension which were both incorporated in the eGFR formula and could be more predictive of the severity of atherosclerosis than the single time point determination of plasma glucose and triglyceride within the TyG index. Future studies should be performed to define the role of the eGDR formula in predicting the burden of atherosclerosis.

The phenotype of T2D in Asians is characterized by young age at the time of onset, predisposition to beta-cell failure, and visceral adiposity even if they do not reach the BMI cut-offs for overweight or obesity in non-Asian populations.[33] Clinical markers that improve the earlier detection of IR would allow the targeting of intensive treatments with lifestyle changes and early uses of insulin-sensitizing medications to those most likely to benefit. Even in an Asian population, there is heterogeneity in the pathogenesis of DM and risks for complications between ethnic/racial groups. In contrast to the South Asian population, lean Thai people with T2D have insulin secretion as a primary defect as stated in a previous euglycemic clamp study done in people with newly diagnosed T2D.[34] The presence of IR received little attention in people with a long-standing duration of T2D. Increased IR was noted not only in people with increased adiposity, hypertension, and dyslipidemia, but also in people with frailty.[7] Additional validated cohorts in the Southeast Asian population with long-standing DM should be conducted to clarify the roles of both the TyG index and eGDR as risk enhancers for reclassifying the risk of individual patients.

Several limitations could have influenced our results. First, the limitations related to the cross-sectional nature of this study and the limited sample size from a specialized diabetes center in Thailand should be considered. The causal relationship between various simple insulin indices and clinical outcomes needs to be confirmed in future prospective studies. Second, the possibility of residual confounding factors cannot be completely ruled out which could affect our results. Other confounding factors like socioeconomic status, physical inactivity, family history, frailty, inflammatory diseases, and environmental exposure were not considered in our study. Third, the cut-off values for HOMA-IR varied greatly from 2.0 to 3.6 in several previous studies based on different geographical populations and studied cohorts.[35-37] However, our study confirmed the significant association between the TyG index and eGDR with the concept of HOMA-IR as reported in previous studies.[12,13] There are no standardized diagnostic criteria or methods to define IR from the HOMA-IR and the defined criteria depend on factors such as age, sex, ethnicity, and clinical conditions.[38] It should be interpreted with caution when extrapolating our findings to other populations. Finally, the limitation of HOMA-IR in participants who were on insulin should be acknowledged. Exogenous insulin might interfere with endogenous insulin secreted into the portal circulation. However, it is still possible to use HOMA-IR to assess insulin sensitivity in subjects treated with insulin as previously mentioned.[39]

CONCLUSION

The TyG index was a useful simple marker for identifying the presence of IR in Thai people with T2D. While the TyG index integrated only fasting glucose and triglyceride levels, eGDR combined other IR factors. Our study demonstrated that the TyG index demonstrated more predictive utility in identifying IR than eGDR. Future longitudinal studies are warranted to demonstrate the potential prediction value of cardiovascular morbidity and mortality for these markers.

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Statement of Authorship
All authors are certified in fulfillment of ICMJE authorship criteria.

CReDIT Author Statement
WC: Conceptualization, Software, Formal analysis, Data Curation, Visualization; **YT:** Methodology, Validation, Investigation, Writing – original draft preparation; **SN:** Resources, Project administration; **EW:** Writing – review and editing; **SK:** Writing – review and editing; **TH:** Supervision, Funding acquisition

Data Availability Statement
Datasets analyzed in the study are under license and not publicly available for sharing.

Author Disclosure
The authors declared no conflict of interest.

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ORIGINAL ARTICLE

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Epidemiologic Profile and Clinical Outcomes of Patients with Pheochromocytoma at the University of the Philippines Philippine General Hospital (UP-PGH)

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Abstract

Objective. This study aims to describe the epidemiologic profile and determine the clinical outcomes of patients with pheochromocytoma at the University of the Philippines Philippine General Hospital (UP-PGH).

Methodology. We reviewed the medical records of 30 patients with histopathology-proven, clinical, and biochemical diagnosis of pheochromocytoma. Demographic, clinical characteristics, and clinical outcomes were collected for each patient.

Results. The median age at diagnosis of pheochromocytoma was 37.5 years (IQR 28-55) and the most common metabolic comorbidities were glucose intolerance (60%) and hypertriglyceridemia (23.3%). Majority of the patients were hypertensive (90%). Two third of the patients presented with classic features of pheochromocytoma while the remaining third presented as adrenal incidentaloma. Recurrence was found in 17% of subjects, who were significantly younger (25 years vs 46.5 years P = 0.0229), and had higher rates of bilateral pheochromocytoma (0 vs 75%), p = 0.002). Metastatic pheochromocytoma was found in 10% of the subjects.

Conclusion. Our study demonstrated that patients with pheochromocytoma in our setting exhibit great variability in terms of clinical behavior. Although majority of the patients presented with symptoms related to catecholamine excess, almost one-third of the patients were only incidentally discovered. Incidence of pheochromocytoma recurrence and metastasis in our setting are comparable with current available foreign studies.

Key words: pheochromocytoma, clinical outcomes, recurrence, metastasis

INTRODUCTION

Pheochromocytoma and paraganglioma (PPGL) are rare catecholamine-secreting neuroendocrine tumors mainly derived from chromaffin cells of the adrenal medulla (80–85%), with a minority originating from the sympathetic and parasympathetic ganglion cells (15–20%), respectively.[1] It has an incidence of two to eight per million persons per year and is found only in 0.1% to 1% of patients presenting with hypertension. Its peak incidence occurs in the third to fifth decade of life but is usually diagnosed at a younger age in hereditary cases, such as in multiple endocrine neoplasia syndrome type 2 (MEN 2), von Hippel-Lindau (vHL) syndrome, and neurofibromatosis type 1 (NF-1).[2]

Pheochromocytoma and paraganglioma are considered to be treatable causes of hypertension. Timely recognition and treatment of these tumors are important because most of these tumors hypersecrete catecholamines which can lead to high cardiovascular morbidity and mortality due to risks of uncontrolled hypertension, stroke, and arrhythmias if untreated.[3] It exhibits great variability in terms of clinical behavior which makes its diagnosis challenging. The diagnosis of pheochromocytoma at the asymptomatic stages of the disease has become more frequent in recent years due to an increase in incidental findings. Presently, available local studies of pheochromocytoma are scarce and limited to case reports only. Moreover, to date, there are still no epidemiological studies on pheochromocytoma conducted in the Philippines. Therefore, this study aims to describe the epidemiologic profile and determine the clinical outcomes of pheochromocytoma among patients admitted at the University of the Philippines Philippine General Hospital (UP-PGH). Furthermore, we will compare the clinical, biochemical, and pathologic features of patients with pheochromocytoma in remission

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versus those who developed recurrence after surgery, and lastly compare those with metastatic and nonmetastatic pheochromocytoma.

METHODOLOGY

Study design

This is a retrospective cohort study which included adult patients diagnosed with pheochromocytoma at the University of the Philippines Philippine General Hospital (UP-PGH) admitted from January 1, 2010, until December 31, 2021, using convenience sampling. Our research protocol was approved by the Technical Review Board of the Department of Medicine of the Philippine General Hospital and the Ethics Board of the University of the Philippines- Manila (UPM-REB Registration No. 2021-613-01) to ensure confidentiality of patient's information. A waiver of informed consent was approved since this study only involved a review of charts.

Inclusion criteria

This study involved adults ≥19 years old diagnosed with pheochromocytoma preoperatively using clinical, biochemical and imaging studies, and underwent adrenalectomy from January 1, 2010 - December 31, 2021, patients with confirmed histopathologic diagnosis of pheochromocytoma, and patients with pheochromocytoma diagnosed biochemically and through imaging who did not undergo surgery for any other reasons.

Exclusion criteria

Patients with adrenal metastasis from another primary cancer tumor were excluded while those patients who were lost to follow-up were excluded in the analysis of outcome.

Data collection

Patients included in the study were selected from the in-patient and outpatient census of the UP-PGH Department of Medicine, the database of adrenalectomy from the Division of Urology and adrenal histopathologic database from the Department of Laboratory. Histologically-proven pheochromocytoma and patients with preoperative diagnosis of pheochromocytoma were selected. Patients diagnosed with pheochromocytoma with normal metanephrines were confirmed through histopathologic reports after undergoing adrenalectomy. Medical records were reviewed where clinical and demographic characteristics were collected. Based on the clinical, biochemical, and radiological results, pheochromocytoma was diagnosed preoperatively . Imaging phenotype which included tumor characteristics such as size, laterality, tumor density in the Hounsfield unit and calculated washout on imaging tests performed such as adrenal CT scan, MRI or MIBG scan was collected. All the operative technique reports and intraoperative monitoring data were collected to determine the surgical approach and intraoperative morbidities. The final histopathologic report including immunohistochemical stain results, if done, was obtained and described based on tumor stage, grade and histology. Analysis of outcome only included patients who underwent surgery while patients who did not undergo surgery were only included in the analysis of clinical characteristics. The outcome of each patient was determined by doing a thorough review of inpatient and outpatient notes, biochemical tests, and imaging results on their follow-up after adrenalectomy. The outcomes measured include biochemical and/or structural cure, biochemical and/or structural recurrence, metastasis, and death. In addition, blood pressure and glycemic control after surgery were also determined to check for complete or partial resolution. Patients with missing data in outcome measures were only included in the clinical profile analysis.

Statistical analysis

Quantitative variables were summarized using the mean and standard deviation (SD) or median and interquartile range (IQR), depending on their distribution. Qualitative variables were tabulated as frequencies and percentages.

Independent samples t-test was used to compare the means of quantitative demographic and clinical variables between patients with and without clinical outcomes of interest (e.g., presence of metastasis). The normality assumption of the t-test was checked using the Shapiro-Wilk test. When the normality assumption was violated, the Mann-Whitney U test was used to compare the distribution of quantitative variables by the presence of clinical outcomes. The chi-square test was used to compare the distribution of qualitative demographic and clinical variables. When the sample size requirement for the chi-square test was not met, Fisher's exact test was used instead. The level of significance for the hypotheses tested was set at 5%. Missing observations were not imputed. Data analysis was performed using Stata SE version 13.

RESULTS

A total of 30 patients with clinically and histologically-confirmed pheochromocytoma admitted at the University of the Philippines Philippine General Hospital (UP-PGH) were included in the study. The median age of diagnosis was 37.5 years (IQR 28-55) and majority were female (70%). The most common comorbidities were glucose intolerance (prediabetes and diabetes mellitus) found in 60% of patients followed by hypertriglyceridemia (23.3%). Majority were hypertensive (90%), while 10% had normal blood pressure at diagnosis. One-third of the patients presented with target organ damage related to chronic hypertension such as ventricular hypertrophy (20%), hypertensive retinopathy (10%) and stroke (3.3%). The classic features of pheochromocytoma (triad of palpitations, diaphoresis and headache) were identified in 66.7% of the patients, and 33% of the patients were diagnosed incidentally through imaging.

Table 1. Demographic and clinical characteristics of the patients with pheochromocytoma admitted in the University of the Philippines Philippine General Hospital between 2010 to 2021 (N = 30)

Characteristics	n (%)
Age at onset (years), median (IQR)	37.5 (28-55)
Duration of symptoms (years), median (IQR)	2 (1-4)
Follow-up (months), median (IQR)	56.0 (24.7-73.8)
Females	21 (70%)
Underwent genetic testing	4 (13.3%)
Comorbidity	
Glucose intolerance	18 (60%)
Hypertriglyceridemia	7 (23.3%)
Left ventricular hypertrophy	6 (20%)
Hypertensive retinopathy	3 (10%)
Stroke	1 (3.3%)
Clinical presentation	
Classic triad of pheochromocytoma	20 (66.7%)
Incidental finding	10 (33.3%)
Anxiety/Panic attacks	7 (23.3%)
Tumor compression symptoms	7 (23.3%)
Orthostatic hypotension	2 (6.7%)
Cardiac arrhythmias	2 (6.7%)
Stage of hypertension	
Normotensive	3 (10%)
Stage 1	6 (20%)
Stage 2	15 (50%)
Resistant Hypertension	6 (20%)
Number of antihypertensive medications before surgery	
0	3 (10%)
1	1 (3.3%)
2	15 (50%)
3 or more	11 (36.7%)
Drug classes of antihypertensive medications	
Alpha blockade	24 (80%)
Beta blockade	20 (66.7%)
CCB	20 (66.7%)
ACE/ARBs	11 (36.7%)
MA	4 (13.3%)
Surgical approach	
Open	21 (75%)
Laparoscopic	3 (10.71%)
Converted	2 (7.14%)
No surgery	2 (7.14%)
Perioperative morbidity	
Hypertensive spikes	18 (72%)
Hypotension requiring vasopressor	9 (36%)
Hyperglycemia	4 (16%)
Laterality	
Unilateral	26 (86.67%)
Bilateral	4 (13.3%)
Clinical outcome	
Recurrence	4 (17.4%)
Remission	19 (82.61%)
Metastasis	3 (10%)
Mortality	2 (8%)

Table 2. Biochemical, radiologic and pathological characteristics of the patients with pheochromocytoma admitted to the University of the Philippines Philippine General Hospital between 2010 to 2021 (N = 30)

Characteristics	n (%)
Tumor diameter (cm), mean (SD)	7.0 (4.0)
Tumor density (unenhanced Hu), mean (SD)	34.7 (5.6)
Level of catecholamine excess from the ULN, median (IQR)	3.46 (2.04-5.85)
Level of catecholamine excess from the ULN	
Normal (less than 2x)	6 (22.2%)
2-5x above ULN	12 (44.4%)
5-10x above ULN	4 (14.8%)
More than 10x above ULN	5 (18.5%)
Tumor diameter (cm)	
<4 cm	5 (17.2%)
≥4 cm	24 (82.8%)
Imaging characteristics	
Heterogenous	14 (46.7%)
Necrosis	10 (33.3%)
Calcification	5 (16.7%)
Absolute wash out ≤60%	6 (60%)
Relative wash out ≤40%	7 (70%)
IHC staining	
Synaptophysin	12 (85.7%)
Chromogranin	14 (100%)
S-100	10 (66.7%)

Most of the subjects required two (50%), and three or more antihypertensive medications (36.7%) for blood pressure control. About 80% of the patients were given alpha blockade and 66.7% received beta blockade. Open adrenalectomy was performed in most of the patients because this has been the standard approach of adrenalectomy in our setting during the earlier years, and also due to larger tumor size observed in our cohort. Intraoperative blood pressure spikes (72%) were the most common perioperative morbidity. Only four patients (13.3%) underwent genetic testing for which three had positive germline mutations and all presented with bilateral pheochromocytoma. Two of the bilateral pheochromocytomas presented synchronously while the other two presented in a metachronous fashion. Four patients (17.4%) experienced recurrent pheochromocytoma. Two patients included in this study died before their scheduled surgery (Table 1).

In our institution, 24-hour-urine metanephrines and plasma metanephrines were the most common screening tests utilized and performed in 91% of tested patients. The patients' median catecholamine elevation was 3.46 times higher than the upper limit of normal values with the majority having more than two times elevation. Nevertheless, 22.2% of the patients had normal metanephrine levels. In terms of imaging phenotype, the mean tumor size was 7 cm with 82.8% of the patients having a tumor size of more than 4 cm. The mean tumor density was 34.7 Hounsfield units (Hu) and an SD of 5.6 Hu on unenhanced CT. Heterogenous enhancement (46.7%) and necrosis (33.3%) were commonly observed among these patients (Table 2).

Compared to patients with clinically suspected pheochromocytoma, those who were incidentally diagnosed through imaging were significantly older (56.4 years vs. 32.6 years, p <0.001) and had a lower prevalence of hypertension (80% vs. 95%, p = 0.005). Patients with clinically suspected pheochromocytoma had significantly higher systolic (195.5 mm Hg vs. 129 mm Hg, p <0.001) and diastolic blood pressure (106.5 mm Hg vs. 78 mm Hg, p = 0.003) at diagnosis compared to those incidentally diagnosed. However, tumor size and metanephrine elevation were not significantly different between the two groups (Table 3).

Of the twenty-three patients with available clinical outcomes, four patients (17.4%) experienced recurrent pheochromocytoma. The mean age of patients with recurrent pheochromocytoma was significantly lower than patients

Table 3. Comparison of patient's demographic and clinical characteristics according to the circumstance of the diagnosing pheochromocytoma

Characteristics	Circumstance of diagnosis		*p*-value
	Incidental (n = 10), mean (SD)	Clinically suspected (n = 20), mean (SD)	
Age at diagnosis (years) [a]	56.4 (10.4)	32.6 (13.0)	<0.001
Tumor size (cm) [a]	7.2 (2.8)	6.9 (4.6)	0.839
Systolic blood pressure upon diagnosis (mm Hg) [a]	129 (17.3)	195.5 (40.3)	<0.001
Diastolic blood pressure upon diagnosis (mm Hg) [a]	78 (7.9)	106.5 (20.8)	0.003
Level of elevation of metanephrine, median (IQR) [b]	2.6 (0-3.9)	3.8 (3.06-10.2)	0.066
Female gender, n (%) [c]	6 (60%)	15 (75%)	0.431
Prevalence of hypertension, n (%) [c]	8 (80%)	19 (95%)	0.005

[a] Independent samples t-test, [b] Mann-Whitney Test, [c] Fisher's exact test

Table 4. Comparison of the demographic and clinical characteristics of PPGL according to recurrence of pheochromocytoma

Characteristics	Recurrent pheochromocytoma		*p*-value
	Without (n = 19), n (%)	With (n = 4), n (%)	
Age (years), mean (SD) [a]	46.5 (16.8)	25 (9)	0.023[a]
Tumor diameter (cm), median (IQR) [b]	7.2 (5-9)	8.35 (5.9-10.6)	0.393[b]
Females [c]	15 (79.0%)	3 (75%)	1.000[c]
Bilateral pheochromocytoma [c]	0 (0%)	3 (75%)	0.002[c]
Genetic mutation [c]	0 (0%)	2 (100%)	0.333[c]

[a] Independent samples t-test, [b] Mann-Whitney Test, [c] Fisher's exact test

Table 5. Comparison of the demographic and clinical characteristics of pheochromocytoma with and without metastasis

Characteristics	Metastatic pheochromocytoma		*p*-value
	Without (N = 27), n (%)	With (N = 3), n (%)	
Age (years), mean (SD) [a]	41.7 (17)	30 (12.1)	0.2527
Tumor size (cm), mean (SD) [a]	6.6 (4.1)	10.1 (0.9)	0.1628
Bilateral [b]	3 (11.1%)	1 (33.3%)	0.360
Genetic mutation [b]	2 (66.7%)	1 (100%)	1.000
Tumor more than 5 cm [b]	18 (72%)	3 (100%)	0.551

[a] Independent samples t-test, [b] Fisher's exact test

who did not experience recurrence (25 years vs 46.5 years, $p = 0.0229$). Furthermore, the proportion of patients with bilateral pheochromocytoma was significantly higher among those with recurrent pheochromocytoma compared to those without (75% vs 0%, $p = 0.002$) (Table 4).

Metastatic pheochromocytoma was diagnosed in three patients (10%). There was no significant difference between the clinical and demographic characteristics of metastatic and non-metastatic pheochromocytoma (Table 5). Two patients with metastatic pheochromocytoma underwent resection of metastatic foci while the remaining patient was subjected to systemic chemotherapy because of a more disseminated disease. Two patients subjected to surgery achieved clinical and biochemical remission while the one who received systemic chemotherapy had progressive disease.

Table 6. Clinical outcome of patients with pheochromocytoma after adrenalectomy in University of the Philippines Philippine General Hospital (N = 23)

Clinical outcomes	n (%)
Number of antihypertensive medications before surgery, median (IQR)	2 (2-3)
Number of antihypertensive medications after surgery, median (IQR)	0 (0-1)
Resolution of symptoms related to catecholamine excess	17 (100%)
Within 1 month postoperatively	14 (82.3%)
Within 1-3 months postoperatively	3 (17.7%)
Improvement of glycemic control	11 (78.6%)
Resolution of hypertension	
Partial resolution	3 (15%)
Complete resolution	12 (60%)
Hypertension persistence	5 (25%)

Among the 30 patients in this study, 26 were subjected to adrenalectomy, two patients were still for surgery as of writing, and two patients died before their surgery. One patient had missing data on clinical outcomes, while the other two patients are still for follow-up after their adrenalectomy. All patients presented with symptoms of catecholamine excess experienced resolution of symptoms after surgery with the majority (82.3%) experiencing immediate resolution (<1 month) after surgery. Likewise, there was observed improvement in glycemic control (78.6%) and resolution of hypertension (75%) after surgery in most of the patients. Overall, there was an observed decline in the median number of preoperative antihypertensive medications after surgery (Table 6).

Patients with persistent hypertension after adrenalectomy were significantly older compared to patients whose hypertension was resolved (64 years vs. 34.5 years, $p = 0.0005$). Meanwhile, patients whose hypertension was resolved had significantly higher preoperative mean SBP (190.7 mm Hg vs. 138 mm Hg, $p = 0.0231$) and higher mean DBP (100 mm Hg vs. 80 mm Hg, $p = 0.0207$) compared to

Table 7. Comparison of clinical and demographic characteristics of patients according to the resolution of hypertension of patients operated for pheochromocytoma

Characteristics	Resolution of hypertension		*p*-value
	Persistent (n = 5), mean (SD)	Resolved (n = 15), mean (SD)	
Age (years) [a]	64.2 (2.2)	34.5 (15.3)	0.0005
Tumor diameter cm [a]	6.7 (3.7)	7.3 (2.5)	0.7156
Systolic BP upon diagnosis (mm Hg) [a]	138 (11.0)	190.7 (46.2)	0.0231
Diastolic BP upon diagnosis (mm Hg) [a]	80 (7.1)	100 (16.9)	0.0207
Number of medications preoperatively [a]	2 (0)	2.8 (1.5)	0.2847
Level of cathecholamine excess (from the ULN) [a]	2.2 (2.8)	8.2 (8.6)	0.1945
Bilateral pheochromocytoma, n (%) [b]	0 (0%)	3 (20%)	0.539
Symptoms of cathecholamine excess, n (%) [b]	0 (0%)	12 (86.67%)	0.001

[a] Independent samples t -test, [b] Fisher's exact test

patients with persistent hypertension. A higher proportion of patients with resolved hypertension also had symptoms of catecholamine excess compared to patients with persistent hypertension (86.67% vs. 0%, $p = 0.001$) (Table 7).

DISCUSSION

Major findings of this study demonstrated that the median age of diagnosis of pheochromocytoma was 37.5 years and the most common metabolic comorbidities detected were glucose intolerance and hypertriglyceridemia. Majority of the patients were hypertensive and had classic features of pheochromocytoma, however, about one-third were diagnosed incidentally. Patients with incidental discovery were relatively older and had a lower prevalence of hypertension. The overall recurrence rate was 17%. Those who experienced recurrence were significantly younger and had a higher proportion of bilateral pheochromocytoma. Although most of the patients had resolution of hypertension, about one-fourth of the patients had persistence of hypertension after adrenalectomy and these patients were significantly older. Metastatic pheochromocytoma was found in 10% of the subjects.

In our study, most of the patients have elevated blood pressure upon diagnosis. However, 10% of the patients have normal blood pressure consistent with the findings of Kopetske et al.[4] wherein 6% of patients with pheochromocytoma were normotensive. Some tumors may contain catechol-O-methyltransferase, which is an enzyme capable of converting active catecholamines into inactive metanephrines, which may explain the clinically silent behavior in some patients. These findings emphasize that the absence of hypertension does not rule out the presence of pheochromocytoma.[4]

In recent years, with the advent of modern imaging modalities, widespread use and increased access to cross-sectional imaging, incidental adrenal masses became increasingly detected. This may have contributed to the detection of pheochromocytoma at the presymptomatic stage where patients do not present with the classic features of pheochromocytoma and are detected even in smaller size tumors. In our study, 33% of the patients presented with adrenal incidentaloma. This finding is in line with the study of Kopetske et al.,[4] which postulates the switch in the clinical presentation of modern-era pheochromocytoma, in contrast with the traditional diagnosis based on symptoms.[5] In our study, patients with incidentally detected tumors were significantly older and presented with hypertension and blood pressure peaks less often than patients with clinically suspected pheochromocytoma, which may reflect a difference in tumor biology between the two. These findings highlight the need to establish the clinical thresholds for the diagnosis of pheochromocytoma on a case-by-case basis while taking the patient's presenting style into account. This makes the data important for clinical practice. The detection of adrenal incidentalomas will likely continue to increase, and clinicians should be aware that pheochromocytoma is possible even in adrenal incidentalomas.[4,5]

Surgery is the standard of care for pheochromocytoma.[6] Although pheochromocytoma is considered a treatable cause of hypertension, there is a small proportion of patients without recurrence who remained hypertensive even after surgery. In our study, those subjects with persistent hypertension after surgery were significantly older. One study demonstrated that age and underlying predisposition such as family history of essential hypertension are potential predictors for nonresolution of hypertension. Individuals with increasing age often lose the ability to reverse the structural vascular changes brought about by the excess catecholamine production which may explain the persistence of hypertension.[7]

Hyperglycemia and diabetes mellitus, known to be brought about by excessive catecholamine production, were also observed in around 23-50% of patients with pheochromocytoma. Similar rates of glucose intolerance were observed in our study population. Tumor resection resulted in the resolution of glucose intolerance in 78.6% of patients in the study of Beninato et al.,[8] which was comparable to our study. Hyperglycemia in pheochromocytoma results from inhibited insulin secretion, stimulated glucagon secretion, and increased peripheral insulin resistance which often resolves after adrenalectomy.[9,10]

Overall recurrence rate in our study was consistent with the recurrence rate of 6.5% to 16.5% in other foreign studies.[7,11] This study demonstrated that those with recurrent pheochromocytoma were significantly younger and had a

higher proportion of bilateral pheochromocytoma which was consistent with the study of Caprino et al.[12] This emphasizes the need for a more proactive approach and closer monitoring of these patients for early detection of disease recurrence. Genetic mutation has been shown as a strong independent predictor of recurrence because PPGL associated with genetic mutations are more frequently bilateral or extra-adrenal, associated with multiple synchronous or metachronous tumors and therefore implying a higher risk of recurrence. However, this was not observed in our study and can likely be explained by the low rates of genetic testing and our small sample size.[2,3,12,13]

The incidence of metastatic pheochromocytoma in our study was consistent with current available observational studies. Metastatic pheochromocytoma was seen more among male patients, those with higher plasma norepinephrine levels and urinary metanephrine excretions, and those with larger tumors (more than 5 cm), but this was not observed in our study likely due to our small sample size, and low rates of genetic testing. Certain genetic mutations such as SDHB mutation have been identified to have a higher rate of metastatic disease reaching 30-70%. The rising number of genetic-related PPGLs reaching approximately 40% of cases and the clinical implications of positive genetic testing with the outcome have been one of the bases why recent international guidelines have recommended genetic testing to be routine in all patients with PPGL.[3,12,14]

The main strength of this study is that this is so far the largest cohort of patients with pheochromocytoma conducted in the Philippines which analyzed clinical characteristics and outcomes of pheochromocytoma among Filipinos. Moreover, most of the patients in our study were managed by a multidisciplinary team in a referral center with expertise in adrenal diseases ensuring homogeneous management of this endocrine tumor. Through this study, the clinical profile of Filipinos with pheochromocytoma and their clinical outcomes were explored and better understood. This can help us improve our case detection, and guide in surveillance monitoring of this rare endocrine tumor in our setting, which could translate to early and appropriate management leading to a more favorable prognosis and better outcomes.

This study's limitations include its retrospective nature, which led to typical issues with secondary data, such as missing or incomplete patient information. Additionally, the small sample size reduced the statistical power of our tests, affecting the detection of significant results. Consequently, care should be taken when interpreting the study's non-significant findings. Lastly, only a small number of patients underwent genetic testing, and the absence of other biochemical tests for pheochromocytoma, such as methoxytyramine and chromogranin A, limited our ability to determine specific biochemical phenotypes that might impact clinical presentation and outcomes.

CONCLUSION

Our study demonstrated that patients with pheochromocytoma in our setting have variable clinical behavior. Although the majority of the patients presented with classic symptoms, almost one-third of the patients were incidentally discovered. Clinical outcomes after surgery generally showed improvement of symptoms related to catecholamine excess, and resolution of hypertension and hyperglycemia. Our study also showed comparable rates of remission, recurrence and metastasis with the other foreign studies. Those who developed recurrence were significantly younger and had higher rates of bilateral pheochromocytoma. The low rate of genetic testing in our study is attributed to its limited availability and constraints in cost. Overall, the results of this study provided a better understanding of how these tumors present clinically and of their outcomes, which can help improve case detection and surveillance monitoring tailored to our setting.

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

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

Author Disclosure
Elizabeth Paz-Pacheco is the Editor-in-Chief of JAFES and Cecilia Jimeno is the Vice Editor-in-Chief. They did not participate in the editorial review or decision-making process for this manuscript. Dr. Hernandez has nothing to disclose.

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Datasets generated and analyzed are included in the published article.

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ORIGINAL ARTICLE

Risk Factors for Perioperative Complications, Treatment Outcomes and Aggressive Behavior of the Tumor in Patients with Pheochromocytoma

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Abstract

Introduction. Pheochromocytomas are catecholamine-secreting tumors arising from chromaffin cells of the adrenal gland. Surgery is the only curative treatment with a high biochemical cure rate, low mortality and high risk of perioperative complications.

Objectives. To study the demographic characteristics of patients with pheochromocytoma and to identify the risk factors for perioperative complications, treatment outcomes, and aggressive behavior of the tumor.

Methodology. We retrospectively studied the data of pheochromocytoma patients registered from 2012 to 2022.

Results. In our study, a total of 30 patients with pheochromocytoma were included. The mean age of presentation was 35 ± 12.8 years. Fifty-six percent were females, and the sex ratio was 1.3:1. Pheochromocytoma spells (60%) was the most common complaint, followed by abdominal pain (53%), orthostatic complaints (10%) and incidentalomas (6%). The baseline mean 24-hour urinary total metanephrines was 2963.7 ± 2658 mcg/24 hours, and the mean tumor size was 7.3 ± 0.53 cm. Forty-three percent of patients underwent laparoscopic adrenalectomy, while the rest underwent open surgery. The mean Pheochromocytoma of Adrenal gland Scaled Score(PASS) was 3.41 ± 0.28, and 23% had a high risk for malignancy. Among perioperative complications, hypertensive crisis (17%) was the most common, followed by postoperative hypotension (13%), hypoglycemia (3%) and right-sided pneumothorax (3%). These patients with complications had higher metanephrine levels (5490 vs. 1880 mcg/24 hours, p = 0.001). Blood pressure normalized in 50%, and this was associated with male sex, younger age (29.5 vs. 40 years, p = 0.03), higher metanephrines (4619 vs. 1855 mcg/24 hours, p = 0.001) and smaller tumors (5.91 vs. 8.61 cm, p = 0.046). PASS score greater than or equal to 4 was associated with higher metanephrine levels (5104 vs. 2312 mcg/24 hours, p = 0.021) and larger tumors (9.28 vs. 6.68 cm, p = 0.024). Biochemical cure rate was achieved in 76% of patients after surgery and was associated with older age (37.7 years vs. 27.7 years, p = 0.047) and absence of pheochromocytoma spells (100% vs. 61%, p = 0.014).

Conclusion. Young age, smaller tumor size and higher metanephrine concentrations were associated with normalization of blood pressure post-surgery. On the other hand, older patients and those without pheochromocytoma spells had better biochemical cure rates. Patients with higher baseline metanephrine levels had increased perioperative complications. More aggressive tumor behavior was associated with higher metanephrine levels and larger tumors. Sex, baseline blood pressure and mode of surgery did not have any influence on treatment outcomes.

Key words: Pheochromocytoma spells, metanephrines, perioperative complications, PASS score

INTRODUCTION

Pheochromocytomas are catecholamine-secreting tumors arising from chromaffin cells of the adrenal gland. These are rare tumors seen in 0.1% to 0.6% of patients being evaluated for secondary hypertension.[1] Eighty to eighty-five percent are catecholamine-secreting tumors while the rest are paragangliomas arising from the sympathetic and parasympathetic ganglia.[2] These tumors can be subdivided into adrenergic, noradrenergic and dopaminergic phenotypes based on the predominant catecholamine produced and excreted.[2,3] The incidence of these tumors peaks in the fourth and fifth decades, with equal distribution among males and females. These patients typically present with pheochromocytoma spells- a triad of episodic palpitations, headache, and diaphoresis, with or without hypertension. Only 40% of patients have classic spells, 40% are incidentally detected, and 7 to 18% present with pheochromocytoma crisis, a serious condition characterized by uncontrolled hypertension associated

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with end-organ damage.[4] However, these tumors can present with a plethora of symptoms, as they vary widely in size, catecholamine production and urinary metabolite excretion.[5]

It is essential to suspect, localize, treat and resect these tumors to cure hypertension, reduce cardiovascular morbidity and prevent lethal paroxysms. Surgery is the only curative treatment, but it carries a high risk of massive catecholamine release, causing severe hypertensive crises. Hypotension can occur after tumor resection because of the sudden withdrawal of the pressor action of tumor metabolites.[6] Perioperative complications can occur despite adequate precautions like α-blockade and intravascular fluid replacement and will necessitate prompt medical management. The objectives of this study were to study the demographic characteristics of patients with pheochromocytoma and to identify the risk factors for perioperative complications, treatment outcomes and tumor aggressiveness.

METHODOLOGY

After getting approval from the Institutional Ethics Committee (Certificate No. MC/190/2007/Pt-II/April 2023), we conducted an observational cross-sectional study in our department. We collected the data of patients with pheochromocytoma registered from 2012 to 2022. Patients with incomplete workups, paragangliomas and adrenal tumors secreting other hormones were excluded from the study. The authors confirm the availability of and access to all original data reported in this study.

Study procedure

Any patient with an adrenal tumor and significant elevation in plasma or urinary catecholamines or their metabolites was diagnosed with pheochromocytoma. Patient history, physical examination, biochemical and imaging data were obtained from case records and documented in a structured proforma. The initial biochemical screening test was a 24-hour urinary total metanephrine (metanephrine and normetanephrine) determination by high-performance liquid chromatography – electrochemical detection method. Cases with values over twice the upper limit were considered significant and subjected to anatomical imaging to localize the tumor.

Preoperative stabilization was achieved with α-blockers followed by ß-blockers, adequate hydration and liberal salt intake. Surgical details, histopathology reports and perioperative complications such as hypertension crisis, hypotension, hypoglycemia and other surgical complications were recorded. A repeat 24-hour urinary total metanephrines was done 1 to 2 weeks after surgery. Biochemical cure was defined as the normalization of urinary metanephrines after surgery.

Treatment outcomes like biochemical cure and normalization of blood pressure were assessed in all patients. The risk of malignancy or tumor aggressiveness was graded histopathologically in all patients using the Pheochromocytoma of Adrenal gland Scaled Score (PASS).

Statistical analysis was done using SPSS version 22. Descriptive statistics were used to extract the mean and standard deviation of continuous data. The normality of the data distribution was checked for all variables by using the Shapiro-Wilk test. An independent t-test was used to compare the means of continuous normally distributed data; otherwise, the Mann-Whitney U test was used for non-normal data distributions. The Chi-Square test was used to compare two categorical variables. In Table 1, patient baseline data is divided and compared between male and female groups using the appropriate statistical tests. Perioperative complications, biochemical cure, normalization of blood pressure post-surgery and PASS score were labeled as outcome or dependent variables. Patient baseline characteristics were considered independent variables. In Tables 2 and 3 we used the Chi-Square test and independent t-test to check for any association between independent and outcome variables. A p-value less than 0.05 is considered statistically significant.

RESULTS

Our study included 30 patients with pheochromocytoma after detailed records screening. The mean age of presentation was 35 ± 12.8 years (13 to 56 years). Most of them (70%) were diagnosed in the third, fourth and fifth decade; 20% (n=6) were younger than 18 years and 10% (n = 3) were older than 50 years. Of the 30 patients, 56% were females, with a female-to-male ratio of 1.3:1. The age of presentation was similar between both sexes. Pheochromo-cytoma spells were the most common presenting complaint (60%), followed by abdominal pain (53%), orthostatic complaints(10%) and incidental detection (6%) by imaging. Pheochromocytoma spells and abdominal pain were more common in females than males. Orthostatic symptoms and incidentalomas were predominantly seen in females and males, respectively. Patients with pheochromocytoma spells were younger (31.94 vs 40.27 years, p = 0.04) than those without the classic spell. On examination, 33% of patients were normotensive, 17% had paroxysmal hypertension, and 50% had sustained elevated blood pressure(Table 1). Blood pressure, body mass index(BMI) and glycemic status were similar between males and females. Syndromic associations were found in four subjects(13.33%). Two had features of MEN2B; one patient had neurofibromatosis, and the other was diagnosed with Von Hippel-Lindau syndrome.

The baseline mean 24-hour urinary total metanephrine level was 2963.7 ± 2658 mcg/24 hours (174 to 8838 mcg/24 hrs). Four patients had a noradrenergic phenotype, and the rest were adrenergic. Females had statistically significantly higher baseline values (3709.6 ± 3103 mcg/24 hours) than males (1879 ± 1560 mcg/24 hours). On imaging, the left

adrenal (60%) was more commonly involved than the right (35%), while one patient had bilateral adrenal involvement. The mean size of the tumor was 7.3 ± 0.53 cm (3-13 cm). Females had larger tumors than males, but this was not statistically significant (7.37 vs 7.1 cm, $p = 0.08$). In our study, 13 patients (43%) underwent laparoscopic adrenalectomy, and the rest underwent open surgery. Postoperative mean urinary metanephrines were 522 ± 166 mcg/24 hours and similar in both sexes. The mean PASS score was 3.41 ± 0.28, significantly higher in females than males. Overall, 23% had a high risk of malignancy based on PASS score and was similar in both sexes (Table 1).

Perioperative complications were documented in 33% of our patients. Hypertensive crisis was the most common (17%), followed by post-operative hypotension (13%), hypoglycemia and right-sided pneumothorax. Surgical wound site complications were seen in 3 patients. Complication occurrence was similar between males and females. Patients with perioperative complications had higher baseline metanephrine levels (5490 vs 1880 mcg/24 hours, $p = 0.001$). Other factors like age, sex, pheochromocytoma spells, tumor size and mode of surgery did not show significant association with complications (Table 2).

Treatment outcomes (Table 3)

Normalization of blood pressure – Blood pressure was normalized in 50% of 20 hypertensive patients after surgery. These patients were predominantly male, younger (29.5 vs. 40 yrs, $p = 0.03$), had higher metanephrine levels (4619 vs. 1855 mcg/24 hours, $p = 0.001$) and smaller tumor size (5.91 vs 8.61 cm, $p = 0.046$).

A biochemical cure rate was achieved in 76% (n = 23) of patients at 1 to 2 weeks post-surgery. Most patients who achieved biochemical cure were older (37.7 years vs. 27.7 years, $p = 0.047$) and without pheochromocytoma spells (100% vs. 61%, $p = 0.014$). Our study found no other significant associations between other baseline factors and biochemical cure rate.

Table 1. Demographic and perioperative characteristics of study subjects

Patient factors		Total N = 30 (%)	Male n = 13 (43.3%)	Female n = 17 (56.67%)	*p*-value
Age (years)		35 ± 12.8	34.15 ± 14	35.5 ± 12	0.39
Clinical features					
1. Pheo spells		18 (60%)	5 (28%)	13 (72%)	0.035
2. Pain abdomen		16 (53%)	6 (38%)	10 (62%)	0.491
3. Orthostatic symptoms		3 (10%)	0	3 (100%)	0.110
4. Incidentaloma		2 (6%)	2 (100%)	0	0.094
Patients with blood pressure	1. Sustained hypertension	15 (50%)	6 (40%)	9 (60%)	0.873
	2. Paroxysmal hypertension	5 (17%)	2 (40%)	3 (60%)	
	3. Normal blood	10 (33%)	5 (50%)	5 (50%)	
BMI (km/m²)		19.2	20.5	18	0.93
Patients with diabetes mellitus		9 (30%)	3 (33.33%)	6 (66.66%)	0.07
HBA1c (%)		8.2 ± 1.2%	8.2 ± 1%	8.6 ± 0.7%	0.87
24-hour urinary total metanephrines (mcg/24 hrs)	Pre-op	2963.7 ± 2658	1879 ± 1560	3709.6 ± 3103	0.022
	Post-op at 2 weeks	522 ± 911	290 ± 176	699 ± 1185	0.08
Size of tumor (cm)		7.3 ± 0.53	7.1 ± 0.68	7.37± 0.68	0.43
PASS score	Total mean score	3.41 ± 0.28	2.84 ± 0.4	3.82 ± 0.37	0.04
	Low risk <4 (n)	23 (77%)	11 (48%)	12 (52%)	0.368
	High risk ≥4 (n)	7 (23%)	2 (28.5%)	5 (71.5)	
Biochemical cure achieved		23 (76%)	8 (35%)	15 (65%)	0.087
Patients with perioperative complications	Total patients	10 (33.33%)	5 (50%)	5 (50%)	0.12
	HTN Crisis	5 (17%)	3 (60%)	2 (40%)	0.45
	Hypotension	4 (13%)	2 (50%)	2 (50%)	-
	Hypoglycemia	1 (3%)	0	1	-
	Surgical complications	3 (10%)	2 (66.66%)	1 (33.33%)	-

Table 2. Association between patient characteristics and perioperative complications

Patient factors		Perioperative complications		*p*-value
		Yes (n=10)	No (n=20)	
Age (years)		29.44	37.23	0.078
Sex	Male (n=13)	5 (38%)	8 (62%)	0.314
	Female (n=17)	5 (30%)	12 (70%)	
Pheo spells (n=18)		5 (28%)	13 (72%)	0.538
Size (cm)		6.33	7.7	0.09
Urinary metanephrines (mcg/24 hrs)		5490	1880	0.001
PASS score		3.11	3.52	0.22
Type of Adrenalectomy	Open (n=17)	5 (30%)	12 (70%)	0.49
	Laparoscopy (n=13)	4 (31%)	9 (69%)	

Table 3. Association between patient characteristics and outcome variables

Patient factors		Normalisation of blood pressure in hypertensive patients (n=20)			Risk of malignancy			Biochemical cure achieved		
		Yes (n=10)	No (n=10)	*p*-value	High (n=7)	Low (n=23)	*p*-value	Yes (n=23)	No (n=7)	*p*-value
Age (years)		29.5	40.1	0.03	38.58	33.79	0.84	37.7	27.7	0.047
Sex	Male (n=13)	6 (75%)	2 (25%)	0.068	2 (15%)	11 (85%)	0.173	8 (61%)	5 (39%)	0.374
	Female (n=17)	4 (33%)	8 (67%)		5 (29%)	12 (71%)		15 (88%)	2 (12%)	
Pheo spells	Yes (n=18)	6 (43%)	8 (57%)	0.329	5 (28%)	13 (72%)	0.481	11 (61%)	7 (39%)	0.014
	No (n=12)	4 (66%)	2 (44%)		2 (17%)	10 (83%)		12 (100%)	0	
Urinarymetanephrines (mcg/24 hrs)		4619	1855	0.001	5104	2312	0.021	2875	3254	0.374
Size of tumor (cms)		5.91	8.61	0.046	9.28	6.68	0.024	7.29	7.27	0.493

Risk of malignancy by PASS score – In our study, 23% of patients had a high risk of malignancy (PASS score greater than or equal to 4). This was associated with higher metanephrine levels (5104 vs 2312 mcg/24 hours, $p = 0.021$) and larger tumor sizes (9.28 vs 6.68 cm, $p = 0.024$).

DISCUSSION

In our study, 86% of all tumors were sporadic, and four patients(14%) had features of genetic syndromes [i.e., MEN-2B(2), Neurofibromatosis (1), and VHL (1)]. According to previous studies, 80 to 85% of chromaffin-cell tumors are pheochromocytomas, and 15 to 20% are paragangliomas. One-third of these tumors had syndromic associations.[1] Similar to previous studies, most of our patients were diagnosed in their third to fifth decade, with equal occurrence among males and females.[2] Pheochromocytoma spells were common in females, younger patients, and higher metanephrine levels. Baseline characteristics and outcomes did not differ significantly between normotensive and hypertensive patients. Normotensive patients are either in the presymptomatic stage of the disease or have nonsecreting tumors.[8]

Surgery and complications

Minimally invasive surgery is the treatment of choice for small, solitary intra-adrenal pheochromocytomas without any malignant radiologic features. Otherwise, open surgery is preferred. A review by Araujo et al., on preoperative and anesthetic management recommends strict blood pressure and heart rate control, and blood volume optimization to reduce the risk of perioperative complications.[6] All our patients achieved target blood pressure, heart rate and adequate hydration before surgery. In the latest review of 40,363 post-adrenalectomy patients, surgical outcomes like complications, pulmonary compromise and length of hospital stay were better with laparoscopic adrenalectomy as compared to open surgery.[9] We did not find significant differences in complications based on the type of surgery. The key to success depends on the surgeon's expertise in handling the tumors and appropriate management of complications by the medical team to reduce morbidity.

In our study, 33.3% of patients had tumor-related perioperative complications. We found that complications were associated with higher levels of metanephrines. In similar studies, established risk factors for hemodynamic instability were high plasma catecholamines, larger tumor size (larger than 4 cm), and blood pressure fall of >10 mm Hg after alpha blockade.[7] Perioperative hypertensive crisis is caused by a massive release of catecholamines while handling the tumor, by anesthetic agents, or stress-induced. Proposed mechanisms of post-operative hypotension are withdrawal of the pressor effect of the tumor, excess use of anti-hypertensives, contracted plasma volume, or surgical blood loss.[10] Among our patients, post-operative hypotension promptly recovered with adequate intravenous fluids and minimal dosage of vasopressors. Postsurgical hypoglycemia occurs due to a rebound increase in insulin secretion, as excess catecholamines suppress insulin secretion. This complication is usually seen in 10% of pheochromocytoma patients who undergo surgery.[11] In our patients with hypoglycemia, this occurred about 6 hours after surgery. The patient was managed with IV dextrose and recovered within 24 hours of surgery.

Treatment outcomes

Normalization of blood pressure after surgery was achieved in 50% of patients in our study. Predominantly, these patients were younger and had higher metanephrines and smaller tumor sizes. The increase in tumor size was associated with failure to normalize blood pressure. Other studies have documented normalization of blood pressure in 75% of patients who undergo surgery, comparable to our study.[1] According to previous studies, persistent elevation of blood pressure post-surgery is due to incomplete resection of the primary tumor, occult metastasis, or underlying essential hypertension. Other possible causes may include excessive intravenous fluids, return of autonomic reflexes, permanent changes in vessel walls and inadvertent ligation of the renal artery.[12] Possible explanations for the low normalization rate of hypertension in our study may be due to relatively older age among these patients and low biochemical cure rates.

Biochemical cure post-surgery: The appropriate timing for post-operative evaluation of metanephrines is still debated. Few guidelines suggest testing after 1 to 2 weeks, while others suggest 2 to 6 weeks after surgery.[2,3] It is reasonable to test before the patient's discharge from the hospital or at least one week after surgery. Failure to

normalize metanephrine levels after surgery may be due to residual tumor or occult metastasis.[13] These patients have to be followed up with anatomical imaging at least 3 to 6 months after surgery.[3] In our study, 76% of patients achieved a biochemical cure. We found that the biochemical cure rate was higher in older patients and those without pheochromocytoma spells.

Risk of malignancy: In previous studies, 10 to 15% of pheochromocytomas are malignant.[14] Currently, there are no definite prognostic markers to predict the malignant behaviour of pheochromocytoma. PASS is a histological algorithm with a structural scoring system to predict aggressive biological behavior. It incorporates 12 histological parameters overexpressed in metastatic cases, and a score greater than or equal to 4 predicts the risk of future aggressive behavior.[15] This score has a high negative predictive value, with a sensitivity of close to 100% and specificity of 75% to identify malignant potential.[16,17] In our study, 23% of patients had PASS greater than or equal to 4, associated with higher metanephrines and larger tumor size.

This study has certain limitations. It included a relatively small sample size and was conducted at a tertiary referral center. Hence, most patients were referred due to increased severity and end-stage disease.

CONCLUSION

Pheochromocytomas are rare large tumors with varied clinical presentation, high biochemical cure rates, and low mortality. Young age, smaller tumors and higher metanephrine concentrations were associated with the normalization of blood pressure post-surgery. In our study, older patients and those without pheochromocytoma spells had better biochemical cure rates. Patients with higher baseline metanephrines are predisposed to perioperative complications, whereas both higher metanephrines and large-size tumors were associated with aggressive behavior of the tumor. Sex, blood pressure and mode of surgery may not have any effect on treatment outcomes. Regular annual follow-up is recommended in all postoperative patients.

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

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GN: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization; **DS:** Methodology, Validation, Formal analysis, Resources, Data curation, Writing – original draft preparation, Writing – review and editing, Supervision, Project administration; **UKS:** Validation, Formal analysis, Investigation, Resources, Visualization, Supervision, Supervision; **AB:** Conceptualization, Methodology, Software, Validation, Resources, Data curation, Writing – review and editing, Supervision; **AKB:** Conceptualization, Methodology, Software, Formal analysis, Resources, Data curation, Writing – original draft preparation, Visualization, Supervision.

Author Disclosure
The authors declared no conflict of interest.

Data Availability Statement
Datasets are not publicly available because participants in the study did not give written consent for their data to be shared.

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ORIGINAL ARTICLE

The Glutamate-Serine-Glycine Index as a Biomarker to Monitor the Effects of Bariatric Surgery on Non-alcoholic Fatty Liver Disease

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Abstract

Objective. Bariatric surgery effectively treats non-alcoholic fatty liver disease (NAFLD). The glutamate-serine-glycine (GSG) index has emerged as a non-invasive diagnostic marker for NAFLD, but its ability to monitor treatment response remains unclear. This study investigates the GSG index's ability to monitor NAFLD's response to bariatric surgery.

Methodology. Ten NAFLD participants were studied at baseline and 6 months post-bariatric surgery. Blood samples were collected for serum biomarkers and metabolomic profiling. Hepatic steatosis [proton density fat fraction (PDFF)] and fibroinflammation (cT1) were quantified with multiparametric magnetic resonance imaging (mpMRI), and hepatic stiffness with magnetic resonance elastography (MRE). Amino acids and acylcarnitines were measured with mass spectrometry. Statistical analyses included paired Student's t-test, Wilcoxon-signed rank test, and Pearson's correlation.

Results. Eight participants provided complete data. At baseline, all had hepatic steatosis (BMI 39.3 ± 5.6 kg/m^2, PDFF ≥5%). Post-surgery reductions in PDFF (from 12.4 ± 6.7% to 6.2 ± 2.8%, p = 0.013) and cT1 (from 823.3 ± 85.4 ms to 757.5 ± 41.6 ms, p = 0.039) were significant, along with the GSG index (from 0.272 ± 0.03 to 0.157 ± 0.05, p = 0.001).

Conclusion. The GSG index can potentially be developed as a marker for monitoring the response of patients with NAFLD to bariatric surgery.

Key words: non-alcoholic fatty liver disease, amino acids, metabolomics, bariatric surgery

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) refers to the presence of hepatic steatosis in the absence of other causes of secondary hepatic fat accumulation, such as alcohol consumption. NAFLD encompasses a spectrum of liver pathologies, ranging from benign steatosis in non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH), characterized by inflammation that can lead to fibrosis and cirrhosis. The incidence of NAFLD in Asia is rising and is projected to increase up to 20% within this decade.[1]

Weight reduction remains the primary treatment modality for NAFLD, and bariatric surgery is more effective than lifestyle interventions in combination with the best medical treatment.[2] However, bariatric surgery may be associated with worsening hepatic fibrosis, cirrhosis, and liver failure. Rapid weight loss occurs after bariatric surgery, leading to the mobilization of lipids from peripheral depots and a large influx of free fatty acids (FFAs), which could cause hepatotoxicity.[3] For these reasons, post-bariatric surgery patients need to be monitored for improvements in NAFLD and worsening of hepatic fibrosis.

The glutamate-serine-glycine (GSG) index has been investigated as a novel marker for the severity of NAFLD. This index involves the measurement of glutamate, serine, and glycine, which are precursors of GSH, and is calculated as the ratio of Glutamate/(Serine + Glycine).[4] The GSG index has been studied in both adult and paediatric NAFLD populations. It correlates with the degree of hepatic steatosis and hepatic aminotransaminase levels, independent of traditional risk factors such as adiposity.[4,5] However, the ability of this index to monitor the response of NAFLD to treatment has yet to be evaluated.

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The primary objective of this study is to investigate the ability of the GSG index to monitor the improvement in the severity of NAFLD following bariatric surgery. NAFLD status will be evaluated using multiparametric magnetic resonance imaging (mpMRI) and magnetic resonance elastography (MRE), which measures the degree of hepatic steatosis, fibroinflammation, and fibrosis. We hypothesize that the GSG index decreases in the first 6 months post-bariatric surgery, and the post-surgery change in the GSG index correlates with improvement in hepatic fibroinflammation. Apart from dysregulated amino acid pathways, lipid metabolism is also altered in NAFLD resulting in the accumulation of various intermediates of incomplete lipid oxidation such as acylcarnitines.[6-8] Altered serum acylcarnitine profiles are associated with NAFLD,[9] and this study also aims to explore post-surgery changes in serum acylcarnitines in patients with NAFLD.

METHODOLOGY

Study design and participants

This prospective observational study was conducted at Singapore General Hospital. Ethics approval was obtained from the SingHealth Institutional Review Board (CIRB Ref: 2019/2179), and informed consent was obtained from participants. Participants were eligible for the study if they: (i) were between 21 and 65 years of age, (ii) had a BMI of ≥ 32.5 kg/m^2 with obesity-related complications. Participants were ineligible for the study if they: (i) consumed excessive alcohol (defined as >1 unit/day for females and >2 units/day for males); (ii) had chronic liver disorders other than NAFLD; (iii) took medications that may induce hepatic steatosis; (iv) had contraindications to MRI. All eligible participants under the care of the study team members were invited to participate. Sample size calculation and sampling strategies were not employed due to the exploratory nature of this study.

Participants were assessed at baseline and 6 months post-surgery. Blood samples were collected for serum biochemical analyses and comprehensive metabolomic profiling. mpMRI and MRE were conducted to assess the severity of NAFLD.

Biochemical analyses and metabolomic profiling

Liver function tests, lipid profiles, and serum creatinine, glucose, and insulin levels were analysed with Abbott Architect i200 (Abbott Diagnostics). HbA1c levels were measured with Roche Cobas c501 (Roche Diagnostics). Amino acids and acylcarnitines were measured by liquid chromatography-mass spectrometry (LC-MS). To extract the amino acids, 30 μL of sample was dried and derivatized using phenyl isothiocyanate by incubating at room temperature for 1 hour. The sample was then reconstituted in 5 mM ammonium acetate in methanol and centrifuged to obtain the precipitate. To separate the individual amino acid components, the precipitated protein pellets were diluted with water and analysed on a Waters Acquity UPLC BEH C18 column (1.7 μm, 2.1x50 mm) using a Waters Acquity I-Class liquid chromatography system coupled to a Waters Xevo TQ-XS mass spectrometer (Waters). The liquid chromatography run was performed with 0.2% formic acid in water as mobile phase A and 0.2% formic acid in acetonitrile as mobile phase B. The gradient started at 5% B, before increasing to 12% B at 1.5 minutes, 17.5% B at 2.7 minutes, 50% B at 4 minutes, and 100% B at 4.5 - 5.0 minutes, before returning to 5% B at 5.1 - 5.8 minutes. The flow rate was maintained at 0.8 mL/min except at 4.7 - 5.1 minutes where it was increased to 1.0 mL/min. Temperature of the column was fixed at 50 °C, and the the injection volume at 5 μL. Compounds were ionized in positive mode using electrospray ionization. Data processing was carried out with the Waters TargetLynx software v4.2 (Waters).

For the quantitative analysis of acylcarnitines species, samples were enriched with 10 μL of a deuterium-labelled mixture of acylcarnitines and diluted with 400 μL of methanol. Following centrifugation at 13,000 rpm for 5 minutes at 4°C, the supernatant was collected for analysis. Methanol extracts were erivatised with 3M hydrochloric acid in methanol (Sigma Aldrich) and reconstituted with 80% methanol for LC-MS analysis. Compounds were ionized in positive mode using electrospray ionization. Data acquisition and analysis were conducted using Agilent MassHunter Workstation B.06.00 Software (Agilent Technologies).

Multiparametric MRI

Non-contrast T1, T2*, and proton density fat fraction (PDFF) were acquired using the LiverMultiScan® protocol (Perspectum Ltd., Oxford, UK).[10,11] PDFF measures hepatic steatosis, and iron-corrected T1 mapping (cT1) indicates hepatic fibroinflammatory disease activity. Four transverse slices positioned at the porta hepatis were captured using a shortened modified look-locker inversion (shMOLLI) and a multiecho-spoiled gradient-echo sequence to quantify liver T1 and iron (T2*) fat (PDFF), respectively. During image analysis, cT1 and PDFF maps of the liver were delineated into whole liver segmentation maps using a semiautomatic method. Three 15-mm diameter circular regions of interest were placed on the transverse T2* maps for each slice, covering a representative sample of the liver, to calculate average T2* values for T1 correction. Non-parenchymal structures such as bile ducts and large blood vessels as well as image artifacts were excluded from image analysis.

Magnetic resonance elastography

MRE measures liver stiffness and was performed using a 2-dimensional MRE protocol and interpreted by abdominal radiologists.[12,13]

Body composition

Lean body mass (LBM), fat-free mass (FFM), and fat mass (FM) were measured using dual-energy X-ray absorptiometry (Hologic Discovery Wi densitometer, Hologic, Inc., Massachusetts, USA).

Statistical analysis

The distribution of quantitative data was assessed with the Shapiro-Wilk test. Variables with a normal distribution were expressed as mean ± standard deviation and those with a non-normal distribution as median (interquartile range). The statistical significance of the post-surgery changes in clinical parameters, mpMRI and MRE parameters, serum amino acid and acylcarnitine profiles, and the GSG index were analysed either with the paired Student's t-test (for data following a normal distribution) or the Wilcoxon signed-rank test (for data following a non-normal distribution). Correlations between post-surgery changes in the GSG index with cT1, PDFF, and MRE were examined using Pearson's correlation. Multiple variable analysis was not performed due to the small sample size and exploratory nature of this study. *P*-value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

Two participants did not provide data at all time points and were excluded from the analysis. The characteristics of participants at baseline (5 males, 3 females) are summarized in Table 1. Participants had a mean age of 44.6 ± 9.4 years and a body mass index (BMI) of 39.3 ± 5.6 kg/m^2. All had hepatic steatosis at baseline, defined as PDFF ≥ 5%.

Effects of bariatric surgery on clinical, mpMRI and MRE parameters

All participants underwent laparoscopic sleeve gastrectomy and had their post-surgery follow-up at 21.9 ± 1.9 weeks. There were significant reductions in weight, BMI, fat mass, fat mass percentage, hip and waist circumference, HbA1c, insulin, ALT, GGT, albumin, total protein, PDFF, and cT1. Serum HDL levels increased significantly post-surgery. However, the decrease in MRE liver stiffness measurements (LSM) was not significant.

Effects of bariatric surgery on serum acylcarnitine profiles

Changes in serum acylcarnitine profiles are listed in Table 2. Short-chain acylcarnitines including C2 (12768.7 (12969.3) nM vs. 10032.5 (4857.2) nM, $p = 0.023$), C5 (122.8 ± 19.1 nM vs. 67.6 ± 23.0 nM, $p < 0.001$), and C5:1 (12.6 ± 3.5 nM vs. 7.9 ± 4.9 nM, $p = 0.041$) decreased post-surgery. Medium-chain acylcarnitines including C6 (72.1 ± 18.7 nM vs. 60.8 ± 13.2 nM, $p = 0.042$), C6-OH (48.6 ± 12.7 nM vs. 35.8 ± 7.2 nM, $p = 0.019$) and C12-OH (4.8 ± 1.6 nM vs. 3.1 ± 1.2 nM, $p = 0.019$), and long-chain acylcarnitines including C18:2 (96.5 ± 22.6 nM vs. 84.0 ± 21.6 nM, $p = 0.048$) and C18:3 (8.2 ± 2.7 nM vs. 5.5 ± 1.8 nM, $p = 0.001$) also decreased. On the other hand, serum levels of C18:2-OH (7.0 ± 3.6 nM vs. 10.5 ± 3.9 nM, $p = 0.048$) and C22 (2.7 ± 0.6 nM vs. 3.3 ± 0.9 nM, $p = 0.014$) increased.

Table 1. Baseline and post-surgery characteristics and clinical parameters

	Baseline	Post-surgery	*p*-value
Age	44.6 ± 9.4	-	-
Weight (kg)	106.9 ± 12.1	87.0 ± 12.4	**<0.001***
BMI (kg/m^2)	39.3 ± 5.6	32.0 ± 5.0	**<0.001***
Fat mass (kg)	49.5 ± 14.3	31.0 ± 9.2	**0.002***
Fat-free mass (kg)	57.4 ± 16.2	56.0 ± 10.8	0.723*
Fat mass (%)	46.5 ± 12.8	35.6 ± 9.7	**0.012***
Hip circumference (cm)	127.1 ± 13.8	105.6 ± 13.4	**0.004***
Waist circumference (cm)	117.7 ± 11.8	103.1 ± 11.0	**<0.001***
HbA1c (%)	6.9 ± 1.0	5.9 ± 0.8	**0.009***
Glucose (mmol/L)	6.3 (2.0)	5.3 (1.0)	0.375**
Insulin (mU/L)	16.8 ± 9.3	7.0 ± 4.2	**0.018***
Creatinine	69.8 ± 19.0	66.4 ± 15.7	0.397*
Total cholesterol (mmol/L)	4.0 ± 1.2	4.7 ± 0.6	0.175*
HDL (mmol/L)	1.1 ± 0.3	1.3 ± 0.3	**0.037***
Triglycerides (mmol/L)	1.4 (0.9)	1.2 (0.2)	0.742**
LDL (mmol/L)	2.2 ± 1.0	2.8 ± 0.5	0.154*
Total protein (U/L)	77.1 ± 4.9	71.4 ± 3.8	**0.010***
ALT (U/L)	26.5 (55.5)	21.0 (7.0)	**0.0391****
AST (U/L)	38.9 ± 18.9	25.1 ± 5.6	0.095*
ALP (U/L)	80.6 ± 12.7	76.6 ± 10.1	0.101*
GGT (U/L)	39.5 (12.5)	24.0 (9.5)	**0.008****
Bilirubin (U/L)	9.5 (3.5)	13.0 (6.5)	0.175**
Albumin (U/L)	43.6 ± 2.3	40.6 ± 2.3	**0.011***
PDFF (%)	12.4 ± 6.7	6.2 ± 2.8	**0.013***
cT1 (ms)	823.3 ± 85.4	757.5 ± 41.6	**0.039***
MRE LSM (kPa)	2.3 (0.2)	2.2 (0.3)	0.813**

Data presented as mean ± SD or median (IQR)
SD: standard deviation; IQR: interquartile range; ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; BMI: body mass index; PDFF: proton density fat fraction; cT1: iron-corrected T1; MRE: magnetic resonance elastography; LSM: liver stiffness measurement. Bolded *p* values indicate statistical significance ($p < 0.05$).
*p values obtained by paired Student's t-test
**p values obtained by Wilcoxon signed-rank test

Effects of bariatric surgery on serum amino acid profiles and the GSG index

Changes in the serum amino acid profiles and the GSG index are listed in Table 3. There was a significant reduction in the GSG index post-surgery (0.272 ± 0.03 vs. 0.157 ± 0.05, $p = 0.001$). Aspartic acid (15.7 ± 4.1 vs. 12.1 ± 3.0, $p = 0.037$), glutamic acid (83.0 ± 28.6 vs. 55.9 ± 16.1, $p = 0.014$), phenylalanine (79.0 ± 10.3 vs. 62.8 ± 8.4, $p = 0.002$), tyrosine (78.8 ± 17.3 vs. 64.2 ± 9.7, $p = 0.004$), leucine (192.4 ± 31.7 vs. 121.4 ± 23.7, $p = 0.001$), isoleucine (116.0 ± 16.6 vs. 79.5 ± 19.4, $p = 0.002$), valine (414.7 ± 42.2 vs. 267.0 ± 51.3, $p < 0.001$), and proline (261.3 ± 63.7 vs. 213.2 ± 48.5, $p = 0.005$) also decreased, whereas levels of arginine (113.7 ± 18.3 vs. 126.4 ± 22.4, $p = 0.012$) increased.

Correlation between post-surgery changes in the GSG index, mpMRI and MRE parameters

The post-surgery change in the GSG index and cT1 showed a correlation coefficient of 0.658, although statistical significance was not achieved ($p = 0.076$) (Figure 1). No statistically significant correlations were found between the post-surgery change in the GSG index and MRE LSM ($r = 0.232$, $p = 0.580$) or PDFF ($r = -0.405$, $p = 0.320$).

Table 2. Baseline and post-surgery serum acylcarnitine profiles (nM)

	Baseline	Post-surgery	*p*-value		Baseline	Post-surgery	*p*-value
C2:0	12768.7 (12969.3)	10032.5 (4857.2)	**0.023****	C16:3	7.9 ± 2.8	6.2 ± 2.9	0.082*
C3:0	470.9 (149.7)	313.4 (70.1)	0.148**	C16:2	8.2 (2.2)	7.5 (1.6)	0.313**
C4:0	282.8 ± 66.1	235.9 ± 93.4	0.254*	C16:1	26.2 (21.3)	26.2 (7.9)	0.250**
C5:1	12.6 ± 3.5	7.9 ± 4.9	**0.041***	C16	148.3 ± 24.9	138.7 ± 31.7	0.415*
C5:0	122.8 ± 19.1	67.6 ± 23.0	**<0.001***	C16:3-OH/C14:3-DC	1.6 ± 0.8	1.3 ± 0.6	0.340*
C4-OH	52.9 (163.8)	16.2 (31.4)	0.078**	C16:2-OH	6.8 ± 1.4	5.1 ± 1.5	0.051*
C6	72.1 ± 18.7	60.8 ± 13.2	**0.042***	C16:1-OH/C14:1-DC	7.9 (3.0)	7.3 (1.5)	0.313**
C5-OH/C3-DC	28.1 ± 9.4	20.9 ± 5.5	0.067*	C16-OH	13.2 ± 4.6	10.7 ± 2.3	0.098*
C4-DC,C6-OH	48.6 ± 12.7	35.8 ± 7.2	**0.019***	C18:3	8.2 ± 2.7	5.5 ± 1.8	**0.001***
C8:1	176.7 ± 75.0	115.0 ± 61.4	0.091*	C18:2	96.5 ± 22.6	84.0 ± 21.6	**0.048***
C8	150.7 ± 60.8	148.4 ± 35.6	0.859*	C18:1	181.4 ± 52.9	172.1 ± 26.6	0.643*
C5-DC	52.5 ± 10.9	53.0 ± 11.8	0.897*	C18	38.9 ± 5.6	45.2 ± 9.3	0.062*
C8:1-OH/C6:1-DC	36.9 ± 12.6	33.8 ± 7.2	0.595*	C18:3-OH/C16:3-DC	4.4 ± 1.3	5.0 ± 2.0	0.524*
C8-OH/C6-DC	86.1 ± 39.4	69.3 ± 22.0	0.222*	C18:2-OH/C16:2-DC	7.0 ± 3.6	10.5 ± 3.9	**0.048***
C10:3	34.9 (49.1)	21.6 (11.6)	0.078**	C18:1-OH/C16:1-DC	9.0 ± 4.9	6.7 ± 2.8	0.165*
C10:2	15.0 ± 6.1	13.1 ± 5.9	0.589*	C18-OH/C16-DC	9.0 ± 3.1	6.8 ± 2.2	0.194*
C10:1	126.6 (24.1)	103.2 (46.3)	0.844**	C20:4	5.6 ± 1.6	6.3 ± 2.7	0.495*
C10	241.8 ± 99.4	251.6 ± 54.5	0.698*	C20:3	6.0 (2.2)	7.1 (2.3)	0.863**
C7-DC	20.8 ± 4.4	20.9 ± 5.1	0.630*	C20:2	5.5 ± 2.3	7.2 ± 1.3	0.102*
C8:1-DC	18.3 ± 6.7	22.5 ± 14.6	0.309*	C20:1	7.6 ± 1.2	8.2 ± 2.2	0.510*
C8-DC	47.0 ± 26.3	33.4 ± 11.1	0.083*	C20	4.4 ± 1.2	4.9 ± 1.7	0.340*
C12:2	13.2 ± 5.0	15.2 ± 9.5	0.512*	C20:3-OH/C18:3-DC	2.5 (0.2)	3.1 (1.6)	0.945**
C12:1	107.2 ± 43.7	92.9 ± 33.3	0.424*	C20:2-OH/C18:2-DC	2.1 ± 1.0	3.4 ± 1.5	0.118*
C12	90.9 ± 39.9	74.7 ± 20.2	0.169*	C20:1-OH/C18:1-DC	9.0 ± 5.0	8.0 ± 2.7	0.513*
C12:2-OH/C10:2-DC	6.7 ± 3.4	5.5 ± 1.9	0.419*	C20-OH/C18-DC	8.7 ± 3.3	8.6 ± 2.5	0.970*
C12:1-OH	14.1 (7.7)	11.9 (5.1)	0.742**	C22:5	2.4 ± 0.8	1.8 ± 0.9	0.223*
C12-OH/C10-DC	4.8 ± 1.6	3.1 ± 1.2	**0.019***	C22:4	1.5 ± 0.8	1.9 ± 1.2	0.316*
C14:3	4.9 ± 1.6	3.9 ± 1.8	0.238*	C22:3	0.9 ± 0.6	0.7 ± 0.4	0.529*
C14:2	32.1 (14.2)	26.9 (14.0)	0.229**	C22:2	0.9 (0.5)	0.9 (0.7)	1.000**
C14:1	72.3 (57.0)	66.0 (19.5)	0.461**	C22:1	2.1 ± 0.7	2.5 ± 0.8	0.410*
C14	34.5 ± 11.4	27.6 ± 9.2	0.203*	C22	2.7 ± 0.6	3.3 ± 0.9	**0.014***
C14:3-OH/C12:3-DC	1.2 ± 0.5	1.2 ± 0.6	0.942*	C24	21.4 ± 6.8	21.1 ± 7.5	0.947*
C14:2-OH	5.7 ± 2.1	4.6 ± 2.0	0.096*	C26	30.5 ± 10.0	38.0 ± 7.4	0.104*
C14:1-OH	16.5 ± 4.8	13.3 ± 3.7	0.113*	C28	2.2 ± 0.9	3.0 ± 0.8	0.187*
C14-OH/C12-DC	12.5 ± 4.1	9.7 ± 3.5	0.247*				

Data presented as mean ± SD or median (IQR)
SD: standard deviation; IQR: interquartile range. Bolded *p* values indicate statistical significance ($p < 0.05$).
**p* values obtained by paired Student's t-test
***p* values obtained by Wilcoxon signed-rank test

Table 3. Baseline and post-surgery serum amino acid profiles (umol/L) and the GSG index

	Baseline	Post-surgery	*p*-value*
Glycine	212.9 ± 47.5	249.6 ± 34.3	0.078
Serine	142.4 ± 33.3	134.6 ± 26.0	0.605
Threonine	111.7 ± 30.5	89.3 ± 30.3	0.137
Alanine	495.8 ± 122.7	442.4 ± 118.5	0.155
Aspartic acid	15.7 ± 4.1	12.1 ± 3.0	**0.037**
Asparagine	87.3 ± 12.5	78.0 ± 13.2	0.136
Glutamic acid	83.0 ± 28.6	55.9 ± 16.1	**0.014**
Glutamine	1276.4 ± 79.7	1302.2 ± 211.3	0.764
Histidine	90.8 ± 86.2	8.7 ± 6.1	0.278
Phenylalanine	79.0 ± 10.3	62.8 ± 8.4	**0.002**
Tyrosine	78.8 ± 17.3	64.2 ± 9.7	**0.004**
Tryptophan	58.2 ± 12.1	51.8 ± 6.8	0.153
Leucine	192.4 ± 31.7	121.4 ± 23.7	**0.001**
Isoleucine	116.0 ± 16.6	79.5 ± 19.4	**0.002**
Valine	414.7 ± 42.2	267.0 ± 51.3	**<0.001**
Methionine	29.8 ± 5.6	27.0 ± 4.1	0.232
Arginine	113.7 ± 18.3	126.4 ± 22.4	**0.012**
Ornithine	83.3 ± 27.5	70.6 ± 11.5	0.085
Citrulline	32.5 ± 7.1	35.3 ± 7.6	0.267
Proline	261.3 ± 63.7	213.2 ± 48.5	**0.005**
GSG index	0.272 ± 0.03	0.157 ± 0.05	**0.001**

Data presented as mean ± SD
SD: standard deviation. Bolded *p* values indicate statistical significance ($p < 0.05$).
**p* values obtained by paired Student's t-test

DISCUSSION

The study aimed to investigate the ability of the GSG index to monitor the effects of bariatric surgery in NAFLD patients. We demonstrated that the post-bariatric surgery improvement in hepatic steatosis and hepatic fibroinflammation was significant, along with the reduction in the GSG index.

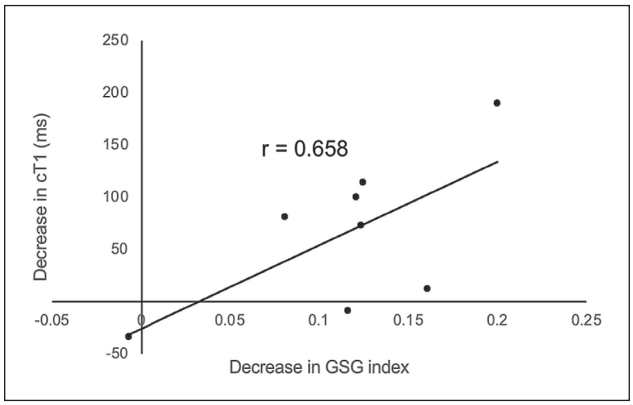

Figure 1. Correlation between the post-surgery decrease in cT1 and the decrease in GSG index.

Bariatric surgery effectively achieves sustained weight loss and can reverse risk factors contributing to NAFLD's pathogenesis, such as dyslipidemia, insulin resistance, and inflammation.[14,15] This concurs with our findings, which demonstrated reduced adiposity and improved lipid and glycemic control. Bariatric surgery also reduces hepatic steatosis, inflammation, and hepatocyte ballooning.[16] Our study similarly showed post-surgery improvements in liver biochemistry, hepatic fat content, and fibroinflammation. However, there was no significant post-surgery change in hepatic fibrosis. This may be because none of the participants had significant fibrosis at baseline and the improvement in fibrosis is slower than steatosis and inflammation.[17,18]

Metabolomic profiling involves analyzing a broad range of metabolites from cellular processes and biochemical pathways, including amino acids, lipids, carbohydrates, nucleotides, organic acids, and various small molecules. Compared to general laboratory parameters, it reflects the current metabolic state of the body more accurately. It can be a sensitive and specific biomarker of NAFLD, and the progression of NAFLD has previously been associated with higher serum acylcarnitines levels.[19] Acylcarnitines, especially medium- and long-chain species, activate proinflammatory signaling pathways that are involved in the pathogenesis and progression of NAFLD.[20-22] Serum acylcarnitine levels also reflect fatty acid oxidation[23] and altered fatty acid oxidation has been linked to hepatic steatosis and insulin resistance and is an important factor behind NAFLD.[24-26] Though not demonstrated in this study, levels of unsaturated long-chain acylcarnitine species such as C14:1 and C18:1 were found to be increased with the progression of fibrosis.[9] Likewise, this may also be attributed to the fact that none of the participants had significant fibrosis at baseline and that improvements in fibrosis are seen over longer periods of time.[17,18]

Liver biopsy is the gold standard for diagnosing and staging liver diseases, including NAFLD. However, biopsies are invasive with significant risks and complications and repeated biopsies to track changes in the severity of NAFLD are challenging to perform in clinical practice. Biopsy results are also subject to sampling error and inter- and intra-observer variability. Laboratory parameters such as blood concentrations of hepatic transaminases are non-invasive indicators of liver function. However, these indirect measurements cannot reliably predict the severity of liver disease.[27] As such, advanced imaging methods such as mpMRI and MRE have been developed as non-invasive tools to diagnose and monitor the progression of NAFLD. We previously showed that improvements in the severity of NAFLD after bariatric surgery can be monitored with mpMRI and MRE.[28] Although these imaging methods have a place in secondary and tertiary care settings, MRI can be costly and may not be widely available, limiting their accessibility for routine clinical care. Therefore, there is a clinical need to develop alternative methods to determine the severity of the condition and monitor the response of NAFLD to treatment in a way that is accurate, non-invasive, and accessible.

The liver participates in protein and amino acid metabolism. NAFLD and NASH are characterized by alterations in pathways involving several aspects of amino acid and lipid metabolism.[29-31] One such pathway is the synthesis of glutathione (GSH),[32,33] an important cellular redox buffer and defender against oxidative stress in hepatocytes.[34] Increased oxidative stress in the liver is associated with liver damage and the progression of NAFLD to NASH. GSH levels have been shown to regulate hepatocyte cell death.[35-37] Thus, measuring GSH levels may serve as a marker of NAFLD. Various methods for measuring GSH include enzymatic assays, high-performance liquid chromatography (HPLC), and mass spectrometry. However, HPLC techniques have poor detection limits for GSH.[38] GSH is also sensitive to oxidation, and careful sample preparation is required for analyses, affecting measurement accuracy and reliability. GSH measurements are also not routinely available in clinical laboratories. Measurements of amino acids related to GSH biosynthesis can be a more reliable method for quantifying GSH status.

Previous studies have demonstrated the role of the GSG index as a marker of the severity of NAFLD, independent of traditional risk factors such as adiposity. The GSG index was previously found to be higher among NAFLD patients and positively correlated with levels of liver enzymes and the degree of hepatic steatosis.[4,5] Our findings extend these earlier observations by demonstrating a reduction in the GSG index post-surgery along with reductions in hepatic steatosis and fibroinflammation. We also found a near statistically significant correlation between the changes in GSG index and hepatic fibroinflammation.

Glutamate and glycine, two amino acids that constitute the GSG index, are independent risk factors for hepatic fibrosis.[39] Glycine functions as a rate-limiting substrate for the synthesis of GSH,[33] and decreased glycine level is associated with altered liver metabolism in patients with hepatic fibrosis.[39] It was also previously demonstrated that plasma glycine concentrations and de novo glycine synthesis increased after bariatric surgery, suggesting impaired glycine synthesis due to obesity-induced insulin resistance in NAFLD.[40] On the other hand, increased levels of glutamate were associated with altered liver metabolism.[39] A previous study also demonstrated an association between glutamate concentrations with GGT. Among other amino acids, glutamate was more strongly associated with the severity of hepatic fibrosis.[4]

Apart from amino acids that constitute the GSG index, branched-chain amino acids (BCAAs) such as leucine, isoleucine, and valine are increased in insulin-resistant states,[4,41] which is a major phenotype in NASH.[42] Higher levels of BCAAs are associated with an increased risk of NAFLD.[43-45] BCAA oxidation reduces after bariatric surgery due to the slower breakdown of body proteins as the ability of insulin to suppress proteolysis is restored.[46-48] Our study also showed that these amino acids decreased post-bariatric surgery.

Moreover, increased levels of aromatic amino acids such as tyrosine and phenylalanine have also been found to be associated with an increased severity of liver diseases.[32,49-51] Phenylalanine is converted to tyrosine in the liver, which is further metabolized. NAFLD patients exhibit increased tyrosine concentrations, which is likely due to impaired hepatic metabolism.[32,51] Our findings are corroborated by another study, which also found that levels of these amino acids are reduced post-bariatric surgery among NAFLD patients.[48]

This is the first study investigating the ability of the GSG index to monitor changes in hepatic steatosis, inflammation, and fibrosis following bariatric surgery. Metabolomics may provide a more sensitive understanding of the metabolic changes associated with NAFLD, and our study illustrates the potential of the GSG index as an accessible and non-invasive biomarker to diagnose NAFLD and monitor its response to various interventions.

Our study is exploratory in nature and has a small sample size with a relatively short duration of follow-up. Future studies with larger sample sizes and regression analysis with the addition of other variables will be needed to validate the effectiveness of the GSG index in monitoring NAFLD treatment response. Future work should also evaluate the utility of these biomarkers over longer follow-up periods. In addition, although liver biopsies were not performed to evaluate the response of NAFLD to bariatric surgery, MRI parameters such as PDFF and cT1 have been shown to correlate with histopathological findings.[52] Our study also recruited NAFLD patients with morbid obesity undergoing bariatric surgery. The ability of the GSG index to monitor the response to other pharmacological or lifestyle interventions will, therefore, also need to be examined.

CONCLUSION

The post-surgery change in the GSG index is in the same direction as the improvements in hepatic steatosis and fibroinflammation. The GSG index can potentially be developed as a marker for monitoring the response of patients with NAFLD to bariatric surgery.

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

CRediT Author Statement
NYYT: Formal Analysis, Writing – original draft preparation, Writing – review and editing; **ES:** Writing – review and editing; **LTEC:** Investigation, Resources, Writing – review and editing; **ASCL:** Writing – review and editing; **CHH:** Writing – review and editing; **AKHE:** Writing – review and editing; **WHC:** Writing – review and editing; **PCL:** Writing – review and editing; **MFT:** Investigation, Resources; **JPEC:** Writing – review and editing; **YMB:** Writing – review and editing; **GBBG:** Writing – review and editing; **JC:** Resources, Writing – original draft preparation; Writing – review and editing; **KVC:** Writing – review and editing; **SHYH:** Writing – review and editing; **JPK:** Writing – review and editing; **HCT:** Conceptualization, Writing – review and editing, Supervision, Project administration, Funding acquisition

Author Disclosure
The authors declared no conflict of interest.

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Datasets analyzed in the study are under license and not publicly available for sharing.

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ORIGINAL ARTICLE

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Initiating or Switching to Insulin Degludec/Insulin Aspart in Adults With Type 2 Diabetes in the Philippines: Results from a Prospective, Non-interventional, Real-World Study

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Abstract

Objectives. Blood glucose levels of the majority of Filipino patients with type 2 diabetes (T2D) remain uncontrolled. Insulin degludec/insulin aspart (IDegAsp) is a fixed-ratio co-formulation of the long-acting basal insulin degludec and the rapid-acting prandial insulin aspart. The real-world ARISE (A Ryzodeg® Initiation and Switch Effectiveness) study investigated clinical outcomes across six countries in people with T2D who initiated IDegAsp. This publication presents the clinical outcomes of the Filipino cohort from a subgroup analysis of the ARISE study.

Methodology. This 26-week, open-label, non-interventional study examined outcomes in adults with T2D initiating or switching to IDegAsp (N=185) from other antidiabetic treatments per local clinical guidance.

Results. Compared with the baseline, there was a significant improvement in glycated hemoglobin at the end of the study (EOS) (estimated difference [ED] −1.4% [95% confidence interval −1.7, −1.1]; *P* <0.0001). Fasting plasma glucose (ED −46.1 mg/dL [−58.2, −34.0]; *P* <0.0001) and body weight (ED −1.0 kg [−2.0, −0.1]; *P* = 0.028) were significantly reduced at EOS compared with baseline. IDegAsp was associated with a decrease in the incidence of self-reported healthcare resource utilization. Adverse events were reported in eight (4.3%) participants.

Conclusions. Initiating or switching to IDegAsp was associated with improved glycemic control, lower body weight, and lower HRU for people with T2D in the Philippines. No new, unexpected AEs were reported.

Key words: insulin aspart; insulin degludec, insulin aspart drug combination; type 2 diabetes

INTRODUCTION

It is estimated that 6.3% of the global population is affected by type 2 diabetes (T2D).[1] In the Philippines, there are an estimated 4.3 million adults aged 20–79 years with diabetes, which equates to a prevalence of 7.1%.[2] Amid a background of increasing overweight, obesity[3] and a genetic predisposition among the Asian population,[4] T2D represents a major cause of morbidity and mortality in the Philippines.[5–7] The increasing prevalence and incidence of T2D poses a significant challenge for the region's healthcare system.[8]

A 2008 study of people with T2D in the Philippines found that mean glycated hemoglobin (HbA1c) levels were 8.0%, and a few individuals (15%) achieved the American Diabetes Association target of HbA1c <7.0%, indicating suboptimal management.[9] As a consequence, healthcare

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resource utilization (HRU) is increased, as people with T2D-related complications are more likely to be hospitalized and have extended hospital stays compared to those without complications.[10]

Efforts to improve access to affordable insulin are ongoing. The Philippine Department of Health (DOH) launched the Insulin Medicine Access Program in 2009.[11,12] This public-private partnership provides insulin to 22 hospitals nationwide. However, as these are mainly city-based hospitals, access remains limited for people living in rural or deprived areas who are unable to travel.[11]

In 2014, the national healthcare insurance company in the Philippines, PhilHealth, implemented new guidelines to improve access to medication for non-communicable diseases, including diabetes.[8] Insurance coverage has been limited to oral antidiabetic (OAD) medication only. Consequently, many people with low to middle income face continued challenges in accessing vital medication when a single insulin pen costs three days' minimum wage.[8,11] The 2019 Universal Health Care Act established the Health Technology Assessment Council, an advisory body to provide recommendations on medicines for government funding. Although there are barriers to including new medications in the Philippine National Formulary (PNF), insulin glargine has recently been included based on a recommendation by the Health Technology Assessment Council.[13,14] The availability of biosimilars will facilitate competitive bidding and help reduce costs.[13] However, research suggests that the availability of diabetes medicines, including those in the PNF, is often low in both public and private medicine outlets,[15] and access to medication may remain an issue for people with T2D.

Disease management is often suboptimal, even among people receiving insulin, and many struggle to maintain blood glucose control.[5,9] Using a co-formulation that simplifies the insulin regimen and improves medication management could promote better glycemic control and improve individuals' health-related quality of life. Early and effective glycemic control is crucial in minimizing the burden of T2D. Therefore, there is an urgent need to overcome the current barriers preventing people with T2D in the Philippines from accessing essential care and medication.

Insulin degludec/insulin aspart (IDegAsp) is a fixed-ratio co-formulation of the long-acting basal insulin degludec and the rapid-acting prandial insulin aspart.[16] The BOOST clinical trial program assessed the efficacy and safety of IDegAsp in participants with T2D. This program has demonstrated the potential for IDegAsp to be used for both insulin initiation and treatment intensification.[17–19] Long-term glycemic control was improved, and non-inferiority was demonstrated with IDegAsp versus biphasic insulin aspart 30 in adults with T2D who were insulin-naïve or inadequately controlled on once- or twice-daily basal, premixed, or self-mixed insulin.[17,18] Improved glucose control was also observed in Japanese adults inadequately controlled with OADs and treated with IDegAsp compared with once-daily insulin glargine.[20] Additionally, the IDegAsp co-formulation yielded similar improvements in glycemic control versus a basal-bolus regimen of separate insulin degludec (IDeg) and insulin aspart (IAsp) injections. This indicates the potential of the IDegAsp co-formulation to provide a simplified alternative to a basal-bolus approach to treatment intensification.[19]

Supporting these clinical trial data are two real-world studies in which switching to IDegAsp from twice-daily premixed insulin (N = 55), intensive insulin therapy (N = 60), or insulin glargine ± prandial insulin (N = 236) was associated with improvement in, or maintenance of, glycemic control and fasting plasma glucose (FPG), and lower daily basal and/or total insulin requirement.[21,22]

The ARISE study investigated glycemic control and other clinical outcomes in a real-world clinical setting across six countries in people with T2D who initiated IDegAsp or switched to IDegAsp from alternative antidiabetic treatment according to local clinical practice (Supplementary Figure S1).[23] The ARISE study has provided the first real-world evidence from the Philippines on the IDegAsp co-formulation. This individual country analysis aims to assess the potential impact of IDegAsp on diabetes management in the Philippines.

METHODOLOGY

Study design and population

Study details have been published previously, but in summary, this was a 26-week, real-world, multi-center, open-label, prospective, non-interventional study examining outcomes in adults with T2D treated with IDegAsp at the discretion of their physician (Supplementary Figure S1).[23–25]

Informed consent was obtained prior to study initiation at the baseline visit. The study consisted of intermediate observational visits in accordance with local clinical practice and an end-of-study (EOS) visit, the first visit within the window from weeks 26–36. The decision to initiate or switch to IDegAsp treatment was taken before study initiation and was independent of the decision to include an individual in the study.

Patients with T2D, fulfilling the inclusion and exclusion criteria of the study, were enrolled in the clinics of participating physicians. Data collection was done between September 2019 and December 2020 from 12 sites across the Philippines.

The study was conducted in accordance with the Declaration of Helsinki 2013. A list of independent ethics committees and institutional review boards that approved the study has been published previously.[23]

Study objectives and endpoints

The primary objectives and endpoints of the ARISE study were published previously.[23] The main objective of this analysis was to evaluate glycemic control and other clinical and safety outcomes after initiating or switching to IDegAsp in the subset of the Filipino population (n = 156 completers, n = 298 recruited initially). The sampling methodology was purposive, with patients recruited at the discretion of their clinician. Assuming a mean change in HbA1c of 0.5% (standard deviation [SD], 1.8%) and a missing HbA1c value at EOS in 25% of participants, a minimum of 139 participants were required to detect an HbA1c difference at 90% power.[23] Descriptive statistics (mean, SD, median and range for continuous variables and proportion for categorical variables) were used to describe participants' baseline characteristics.

The primary endpoint was the change in laboratory-measured HbA1c levels from baseline to EOS. Secondary endpoints included the proportion of participants achieving HbA1c levels <7% at EOS, the proportion of participants achieving HbA1c levels below a predefined individualized treatment target at EOS and change from baseline to EOS in FPG, body weight, and total, basal and prandial insulin dose. Additional endpoints included participant-reported non-severe hypoglycemic episodes (nocturnal and total) occurring within four weeks before IDegAsp initiation and within four weeks before EOS and severe hypoglycemic episodes occurring within 26 weeks before IDegAsp initiation and during the 26-week study period, as defined previously.[23]

Secondary objectives were designed to describe the clinical use of IDegAsp in a real-world setting, including physicians' reasons for initiating or discontinuing treatment. HRU associated with the T2D management and related complications was included as an exploratory endpoint.

Statistical methods

The Philippines full analysis set (FAS) included all eligible participants who gave informed consent and initiated treatment with IDegAsp. The in-study observation period was from the informed consent and treatment initiation visit to study completion (first visit within weeks 26–36). Reasons for not completing the study included withdrawal of informed consent and participant lost to follow-up, deceased, or uncontactable (e.g., closure of study site). The on-treatment observation period was the period in which participants were treated with IDegAsp. Values measured after treatment discontinuation were disregarded.

Statistical tests for the primary and secondary endpoints were performed as two-sided tests with a significance level of 0.05. The analysis was performed using SAS software. No adjustments were made for multiple comparisons. The primary endpoint analysis was conducted with a mixed model for repeated measurements and based on all participants with at least one post-baseline HbA1c measurement using the 'in-study' observation period. Secondary analyses of the primary endpoint were conducted using 'on-treatment' data only. The crude model included baseline HbA1c and time of HbA1c measurement as covariates. The adjusted model included baseline HbA1c, time of HbA1c measurement, age, sex, body mass index (BMI) and previous antidiabetic treatment regimen as covariates. Covariates were included in the model based on apriori knowledge regarding factors that could potentially influence glycemic control. The incidence rates of non-severe, nocturnal and severe hypoglycemia were analyzed using descriptive statistics. Safety data on adverse events (AEs) were also reported using descriptive statistics. HbA1c and FPG were done in local laboratories at the request of the managing clinician. Per routine practice, body weight and insulin dose were evaluated during site visits. Hypoglycemia was self-reported.

RESULTS

Study population demographics and clinical characteristics

The overall study population results have been reported previously.[23] Of the 298 people recruited for the study in the Philippines, 185 switched to or initiated IDegAsp and were included in the FAS. Of these, 156 participants (84.3%)

Table 1. Demographics and clinical characteristics at baseline

	Philippines N = 185
Age, mean (SD)	58.5 (12.2)
Sex n (%)	
Female	111 (60.0)
Male	74 (40.0)
Duration of diabetes (years), mean (SD)	10.8 (7.3)
Body weight (kg)[a], mean (SD)	67.1 (14.1)
BMI (kg/m^2), mean (SD)	26.0 (5.3)
HbA1c (%)[a], mean (SD)	10.2 (2.1)
FPG (mg/dL)[a], mean (SD)	208.0 (84.1)
Antidiabetic treatment, n (%)	
OADs only	83 (48.0)
Premix insulin ± bolus insulin (± OADs)	18 (10.4)
Basal insulin only (± OADs)	57 (32.9)
Basal–bolus insulin (± OADs)	11 (6.4)
GLP-1 RA ± insulin (± OADs)	4 (2.3)
Dose of previous prandial insulin (U), mean (SD)	24.6 (21.2)
Diabetes complications, n (%)	
Diabetic neuropathy	35 (27.3)
Diabetic nephropathy	24 (18.8)
Cardiovascular disease	12 (9.4)
Diabetic retinopathy	12 (9.4)
Peripheral vascular disease	4 (3.1)

Global ARISE study data were published previously.[25] OADs included sulfonylureas, meglitinides, biguanides, thiazolidinediones, dipeptidyl peptidase 4 inhibitors, sodium glucose co-transporter 2 inhibitors, and α-glucosidase inhibitors.

[a]Baseline assessments from ≤12 weeks prior to signing informed consent and initiating IDegAsp treatment.

BMI: body mass index; FPG: fasting plasma glucose; GLP-1 RA: glucagon-like peptide-1 receptor agonist; HbA1c: glycated hemoglobin; N: number of participants in the full analysis set; OAD: oral antidiabetic drug; SD: standard deviation; U: units.

completed the study. Baseline demographics and clinical characteristics are presented in Table 1. At baseline, the mean (standard deviation [SD]) age was 58.5 (12.2) years, HbA1c was 10.2 (2.1) %, body weight was 67.1 (14.1) kg, BMI was 26.0 (5.3) kg/m^2, and duration of diabetes was 10.8 (7.3) years.

Prior to initiating or switching to IDegAsp, 173 participants had received prior anti-hyperglycemic treatment. Of these, 48.0% were receiving OADs only, and 32.9% were receiving basal insulin, 10.4% premix insulin, 6.4% basal–bolus insulin, and 2.3% glucagon-like peptide-1 receptor agonists with or without insulin, with or without OADs.

At treatment initiation, 132 participants (71.4%) received IDegAsp once daily, and 52 (28.1%) received IDegAsp twice daily. One patient had a regimen listed as "other," i.e., neither once- or twice-daily dosing. The most frequently cited reasons physicians gave for switching people with T2D to IDegAsp were to improve glycemic control (95.7%), promote convenience and flexibility in the dosing regimen (30.3%), have fewer injections compared with basal and bolus therapy (29.7%), and lower the risk for hypoglycemia. (23.3%) (Supplementary Table S1). Physicians could report more than one reason for initiation. For the 13 instances where IDegAsp treatment was discontinued, an unacceptable glycemic profile was cited as a reason for one participant. In the remaining 12 instances, reasons were not specified.

Glycemic control

The observed mean (SD) HbA1c, adjusted for covariates, at baseline was 10.0% (2.1%), and the estimated mean (SD) at EOS was 8.5% (0.2%). HbA1c was statistically significantly lower at EOS compared with baseline (estimated difference −1.4% [95% confidence interval {CI} −1.7, −1.1]; *P*<0.0001; Table 2). Similarly, there was a significant reduction in FPG from baseline to EOS (estimated difference −46.1 mg/dL [95% CI −58.2, −34.0]; *P*<0.0001). The proportion of participants with HbA1c levels <7.0% increased from 2.2% (n=4) at baseline to 17.2% (n=23) at EOS. The proportion of

Table 2. Adjusted mixed model for repeated measurements showing change in HbA1C; FPG; body weight; and total, basal, and prandial insulin dose over 36 weeks of IDEGASP treatment in the Philippines

	In-study observation period, N=185	On-treatment observation period, N=185
Change in HbA1c (%)		
Patients analyzed, n	135	135
Observed mean HbA1c at baseline, % (SD)	10.0 (2.1)	10.0 (2.1)
Estimated mean HbA1c at EOS (week 36), % (SE)	8.5 (0.2)	8.5 (0.2)
Estimated mean change, % (95% CI)	−1.4 (−1.7, −1.1), P<0.0001	−1.4 (−1.7, −1.1), P<0.0001
HbA1c less than 7%		
At baseline, n (%)	4 (2.2)	4 (2.2)
At EOS, n (%)[a]	23 (17.2)	23 (17.2)
HbA1c less than pre-defined individual treatment target[b]		
At baseline, n (%)	4 (2.2)	4 (2.2)
At EOS, n (%)[a]	22 (16.4)	22 (16.4)
Change in FPG (mg/dL)		
Patients analyzed, n	129	125
Observed mean FPG at baseline, mg/dL (SD)	206.8 (82.8)	207.6 (83.0)
Estimated mean FPG at EOS (week 36), mg/dL (SE)	161.2 (6.1)	162.2 (6.3)
Estimated mean change, mg/dL (95% CI)	−46.1 (−58.2, −34.0), P<0.0001	−45.5 (−58.1, −32.9), P<0.0001
Change in body weight (kg)		
Patients analyzed, n	148	148
Observed mean body weight at baseline, kg (SD)	67.3 (14.8)	67.3 (14.8)
Estimated mean body weight at EOS (week 36), kg (SE)	67.5 (0.5)	67.5 (0.5)
Estimated mean change, kg (95% CI)	−1.0 (−2.0, −0.1), P=0.028	−1.0 (−2.0, −0.1), P= 0.028
Change in total insulin dose (U)		
Patients analyzed, n	79	-
Observed mean total insulin dose at baseline, U (SD)	38.2 (29.3)	-
Estimated mean total insulin dose at EOS (week 36), U (SE)	39.1 (2.0)	-
Estimated mean change,U (95% CI)	1.2 (−2.7, 5.1)	-
Change in basal insulin dose		
Patients analyzed, n	79	-
Observed mean basal insulin dose at baseline, U (SD)	30.0 (15.7)	-
Estimated mean basal insulin dose at EOS (week 36), U (SE)	26.3 (1.2)	-
Estimated mean change,U (95% CI)	−3.3 (−5.6, −1.0)	-
Change in prandial insulin dose		
Patients analyzed, n	79	-
Observed mean prandial insulin dose at baseline, U (SD)	8.2 (16.8)	-
Estimated mean prandial insulin dose at EOS (week 36), U (SE)	12.9 (1.0)	-
Estimated mean change,U (95% CI)	4.6 (2.6, 6.6)	-

The adjusted model included age, sex, body mass index, and previous anti-hyperglycemic treatment regimen as baseline covariates.

[a] n=134; [b] Categories of pre-defined individual treatment target ranges for HbA1c (%) levels were <6.5, 6.5 to <7.0, 7.0 to <7.5, 7.5 to <8.0, and ≥8.

CI: confidence interval; EOS: end of study; FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; IDegAsp: insulin degludec/insulin aspart; N: number of participants in the Philippines full analysis set; n: number of participants; SD: standard deviation; SE: standard error.

participants achieving HbA1c levels below their predefined individual treatment target increased from 2.2% at baseline (n=4) to 16.4% (n=22) at EOS (Table 2).

Body weight

There was a significant reduction in body weight in the overall study population at EOS compared with baseline (estimated difference −1.0 kg [95% CI −2.0, −0.1]; *P*=0.028; Table 2).

Insulin dose

In insulin-experienced participants, the observed mean total daily insulin dose at baseline was 38.2 (SD 29.25) units (U), and the estimated total daily insulin dose at EOS was 39.1 (SD 17.51) U. There was a significant increase in the observed mean daily prandial insulin dose from 8.2 (SD 16.81) U at baseline to 12.9 (SD 8.98) U at EOS, *P*<0.0001. The mean daily basal insulin dose decreased from 30 (SD 15.66) U at baseline to 26.3 (SD 10.40) U at EOS, *P*<0.05.

Hypoglycemia

The estimated incidence of non-severe, nocturnal and severe hypoglycemic episodes were reduced numerically after treatment initiation (Table 3). Due to the small sample size, these data were not analyzed statistically.

Healthcare resource utilization

For HRU associated with diabetes and its complications, initiating or switching to IDegAsp resulted in a decrease in the incidence of self-reported outpatient visits (26 vs. 7) and inpatient hospitalizations (8 vs. 1) in the 12 weeks prior to baseline versus the 12 weeks prior to EOS or IDegAsp discontinuation. The number of workdays missed in the Philippines cohort decreased from 3 to 0 over the same time period (Table 4 and Supplementary Table S2).

Adverse events

AEs were reported in eight (4.3%) participants in the Philippines cohort. This included four serious AEs in three (1.6%) participants (cerebrovascular disorder, community-acquired pneumonia, COVID-19 and death) and eight nonserious AEs in five (2.7%) participants (abdominal pain, body weakness, abdominal discomfort, paronychia, neck abscess, scrotum abscess, dyslipidemia, hyperuricemia) (Table 5). Three serious AEs and six nonserious AEs were judged as unlikely to be caused by IDegAsp treatment. The remaining two nonserious AEs (abdominal pain and body

Table 3. Summary of hypoglycemic events occurring prior to initiation of idegasp (baseline) and prior to EOS or discontinuation in the Philippines

Hypoglycemic events	Number of events	Number of patients, n (%)
Non-severe	73	16
Within 4 weeks prior to initiation	40	13 (81.3)
Within 4 weeks prior to EOS or discontinuation	33	3 (18.8)
Nocturnal non-severe	12	6
Within 4 weeks prior to initiation	10	5 (83.3)
Within 4 weeks prior to EOS or discontinuation	2	1 (16.7)
Severe	3	2
Within 26 weeks prior to initiation	3	2 (100.0)
Within 26 weeks prior to EOS or discontinuation	0	0

Data based on the full Philippines analysis set.

EOS: end of study; IDegAsp: insulin degludec/insulin aspart; n: number of participants with a response.

Table 4. Summary of HRU prior to initiation of IDEGASP (baseline) and prior to EOS in the Philippines

HRU associated with diabetes and its complications	n	Number of visits/resources used, mean (SD)
Self-reported outpatient visits		
Within 12 weeks prior to initiation	26	1.8 (1.3)
Within 12 weeks prior to EOS or discontinuation	7	1.7 (1.0)
Self-reported emergency room visits		
Within 12 weeks prior to initiation	4	1.0
Within 12 weeks prior to EOS or discontinuation	0	0
Self-reported other healthcare provider visits and contacts outside of the hospital setting[a]		
Within 12 weeks prior to initiation	0	0
Within 12 weeks prior to EOS or discontinuation	5	2.2 (1.3)
Self-reported workdays missed		
Within 12 weeks prior to initiation	3	12.0 (15.7)
Within 12 weeks prior to EOS or discontinuation	0	0
Self-reported inpatient hospitalizations		
Within 12 weeks prior to initiation	8	1.0
Within 12 weeks prior to EOS or discontinuation	1	1.0

[a] Includes face-to-face, telephone, and email.

EOS: end of study; IDegAsp: insulin degludec/insulin aspart; HRU: healthcare resource utilization; n: number of participants contributing to the analysis; SD: standard deviation.

Table 5. Adverse events in Philippines cohort of ARISE

	Serious			Nonserious			Total		
	n	%	E	n	%	E	n	%	E
Adverse events	3	1.6	4	5	2.7	8	8	4.3	12
Severity									
Mild	1	0.5	1	5	2.7	8	6	3.2	9
Moderate	1	0.5	1	0	-	-	1	0.5	1
Severe	2	1.1	2	0	-	-	2	1.1	2
Causality									
Probable	0	-	-	1	0.5	2	1	0.5	2
Possible	0	-	-	0	-	-	0	-	-
Unlikely	2	1.1	3	4	2.2	6	6	3.2	9

Data based on Philippines FAS.

%: percentage of participants; E: number of events; FAS: full analysis set; n: number of participants.

weakness) were judged to be probably caused by IDegAsp treatment (Table 5).

DISCUSSION

This real-world study demonstrates the potential impact of the IDegAsp co-formulation in a clinical setting in the Philippines, where there is a need to improve glycemic control and reduce the financial burden of medication for people with T2D.

A greater proportion of participants in the Philippines were receiving OAD medication only at baseline compared with the global ARISE study (48% vs. 35.1%).[23] This likely reflects the lack of access to insulin therapy in the region, as well as widespread clinical inertia in diabetes care, resulting in delays in treatment intensification.[26,27] In line with our observation, a real-world study of 1065 participants with T2D in the Western Pacific region found that ~66% had an HbA1c level ≥9.0% at the time of insulin initiation despite receiving two or more OADs.[27]

Initiating or switching to IDegAsp was associated with significant reductions in HbA1c and FPG at EOS compared with baseline for participants in the Philippines. The mean change in HbA1c from baseline to EOS was −1.4% for both the global and Philippines analyses.[23] The significant reduction in FPG from baseline to EOS in both the global study and the Philippines cohort following the initiation of IDegAsp reflects the reduction observed in HbA1c. This Philippines substudy was not statistically powered to analyze treatment effect by prior treatment regimen. However, the global ARISE study found the most significant improvement in glycemic control among people previously receiving OAD therapy only.[23] The proportion of participants with HbA1c levels <7.0% was numerically higher at EOS versus baseline for both the global and Philippines analysis sets.[23]

Using high dosages of basal insulin has been associated with an increased risk of hypoglycemia. An analysis of pooled data from 15 randomized trials in insulin-naïve participants with T2D treated with basal insulin glargine, with or without OADs, for ≥24 weeks found that titration of basal insulin to doses >0.5, >0.7 and >1.0 IU/kg did not improve glycemic control and was associated with an increased risk of hypoglycemia when the dose cut-offs were exceeded.[28] It is therefore important that the global ARISE study and Philippines subanalysis found that initiating or switching to IDegAsp improved glycemic control while leading to significant reductions in daily basal insulin dose. For participants receiving premix or basal–bolus insulin prior to the study, switching to IDegAsp was associated with significant reductions in daily total insulin dose. Reductions in insulin dosage are associated with decreased healthcare costs and the risk of AEs. In this study, all AEs reported were similar to those in previous trials of IDegAsp,[16–22] and no new, unexpected AEs were reported in the ARISE Philippines cohort.

While treatment with premixed insulin analogs offers greater convenience than multiple daily basal–bolus injections, interactions between the basal and bolus components of biphasic formulations can potentially result in delayed postprandial hypoglycemia.[29,30] Accordingly, it is remarkable that the observed improvements in glycemic control were attained without any additional risk of severe, non-severe and nocturnal hypoglycemia. These results are supported by a Phase 3 trial of 296 people with T2D in Japan, in which a significantly higher proportion of participants achieved an HbA1c <7% without hypoglycemia with IDegAsp treatment compared with once-daily insulin glargine (43 vs. 25%; estimated odds ratio [IDegAsp/IGlar] 2.21 [95% CI 1.25, 3.92], P<0.01).[20]

The most frequently cited reason physicians gave for switching people with T2D to IDegAsp was to improve glycemic control, preventing the development or progression of comorbidities arising from inadequate control. Additionally, physicians opted to initiate IDegAsp therapy due to flexibility in the dosing regimen and the need for fewer injections compared with basal–bolus therapy. This highlights the potential of IDegAsp co-formulation to minimize treatment burden and overcome clinical inertia that can delay access to appropriate T2D care.[31]

In the Philippines, approximately 27 million individuals are estimated to be overweight or obese, and the prevalence is rising — overweight and obesity almost doubled between 1998 and 2019, increasing from 20.2% to 36.6%.[3] It is, therefore, crucial that T2D treatments do not contribute to this issue. Insulin and OADs, including sulfonylureas, thiazolidinediones, and meglitinides, are associated with weight gain.[32–34] In the ARISE global cohort and Philippines cohort, improvements in glycemic control were achieved alongside significant reductions in body weight.

The reduction in body weight observed in the Philippines cohort could be related to several factors: discontinuation or dose reduction of OADs that induce weight gain; reduced hypoglycemic episodes may have decreased participants' propensity for '*defensive eating,*' thereby leading to reductions in calorie intake; and treatment adherence may

have been improved in this clinical study setting compared with participants' daily routines.

Socioeconomic factors play a major role in the quality and consistency of diabetic care that is accessible in the Philippines.[8,35] A study evaluating access to diabetes care in the Philippines found that people with T2D took their medications intermittently based on their own judgment, selecting some elements of medical advice, and weighing symptoms against medication cost.[35] As highlighted by the results described above, there is a tendency towards delayed insulin initiation in Asia. People with T2D are often receiving multiple OADs when insulin is prescribed.[27] Timely access to insulin therapy is needed to enable optimal glycemic control and prevent comorbidities.[27] The reduction in diabetes-associated HRU observed in those switching to IDegAsp in both the global and Filipino studies demonstrates the potential to reduce the financial burden associated with T2D.[23]

Although efforts have been made to improve care for people with T2D through initiatives such as the Insulin Medicine Access Program, insulin access remains limited for many in the Philippines.[11] Following the enactment of the Universal Health Care act into law in 2019, all Filipinos are automatically enrolled in the national health insurance program.[36] This enables individuals to access health services without increasing their financial burden. While insulin glargine is now included in the PNF, access to medications in the PNF is often low in both public and private medicine outlets.[15] For those who do have access to insulin, management of T2D is often suboptimal.[5,9] Equitable access to treatment options that simplify the insulin regimen and improve medication management could help to improve glycaemic control and quality of life for people living with T2D. We recommend that the HRU data from ARISE be considered by the DOH within this context to assess the impact of IDegAsp on people with T2D.

The limitations of this study have been reported previously.[23] The study design did not allow control over baseline parameter ranges. As an open-label, single-arm study, there was no control group for comparison. Thus, the study effect could not be estimated, and any additional factors, such as changes to other elements of treatment, were not accounted for. Although all participants were recruited prior to the COVID-19 pandemic, follow-up became challenging owing to the strict implementation of health protocols and lockdowns in the country. Several participants were lost to follow-up during this period.

CONCLUSION

Results from this open-label, single-arm, non-interventional study of people with T2D treated with IDegAsp in the Philippines found improved outcomes, including improved glycemic control, lower body weight, and lower HRU following treatment initiation. No new, unexpected AEs were reported.

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

CRediT Author Statement
NNJ: Investigation, Resources, Writing – review and editing; **NAG:** Investigation, Writing – review and editing; **GA:** Investigation, Writing – review and editing; **GJRA:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration; **OAD:** Investigation, Writing – review and editing; **REF:** Investigation, Writing – review and editing; **NTF:** Resources, Writing – review and editing, Supervision, Project administration, Funding acquisition; **SK:** Investigation, writing – review and editing; **BM:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration; **RM:** Investigation, Writing – review and editing; **AP:** Investigation, Resources, Writing – review and editing; **FP:** Investigation, Writing – review and editing; **MPR:** Resources, Writing – review and editing, Supervision, Project administration, Funding acquisition; **AS:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **MT:** Investigation, Resources, Data Curation, Writing – review and editing, Visualization

Author Disclosure
Nicole-Therese Flor, Mercerose Puno-Rocamora, and Ahsan Shoeb are employees of Novo Nordisk Philippines, Taguig City, Philippines and hold stocks in Novo Nordisk.

Data Availability Statement
Datasets generated and analyzed are included in the published article.

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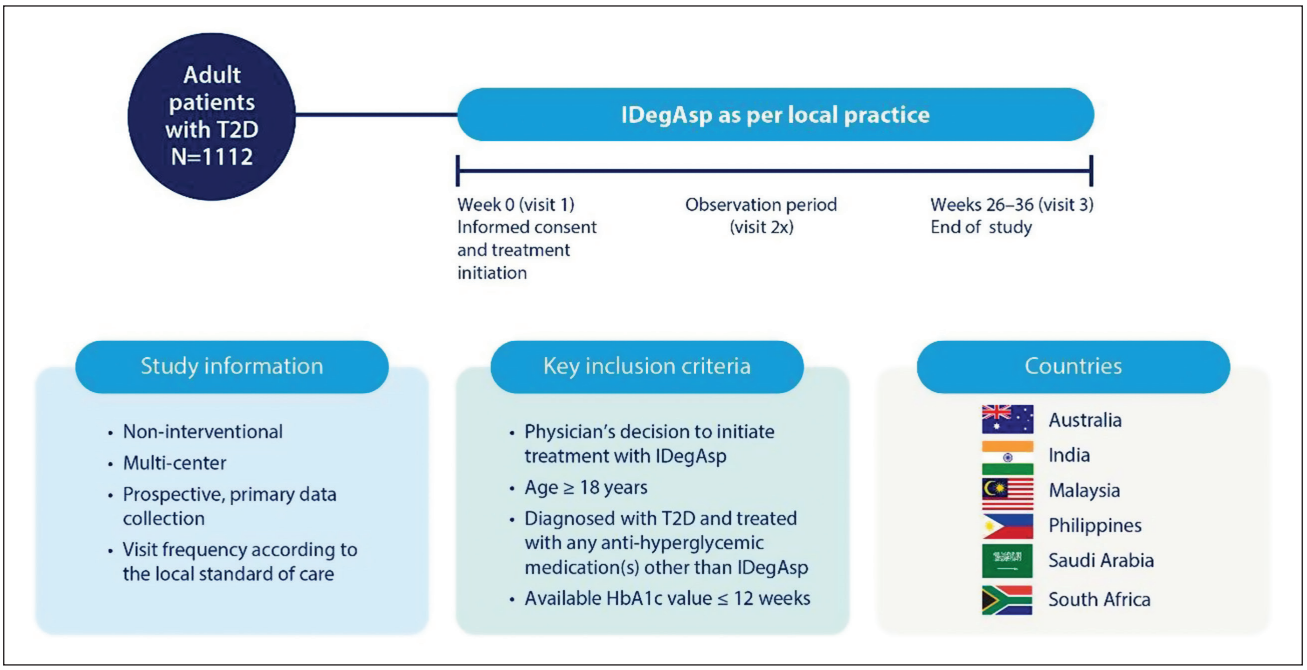
SUPPLEMENTARY MATERIALS



Supplementary Figure S1. Study design.

HbA1c: glycated hemoglobin; IDegAsp: insulin degludec/insulin aspart; N: number of participants enrolled into the full study; T2D: type 2 diabetes.

Supplementary Table S1. Physician explanations for initiating or moving participant to IDEGASP

	n (%) (N=185)
To improve the participant's glycemic control	177 (95.7)
To lower the risk of hypoglycemia	43 (23.2)
Flexibility in the dosing regimen	56 (30.3)
Fewer injections than basal and bolus therapy	55 (29.7)
No reconstitution needed	16 (8.6)
Change in coverage status favoring IDegAsp	12 (6.5)
Other	1 (0.5)

Physicians could select more than one reason for each participant. A change in coverage status favoring IDegAsp refers to a change in healthcare insurance or reimbursement requirements that led to better access to the drug.

IDegAsp: insulin degludec/insulin aspart; n: number of participants; N: number of participants in analysis set.

Supplementary Table S2. Summary of HRU prior to initiation of IDEGASP (baseline) and prior to EOS in the Philippines

HRU associated with severe hypoglycemia	Number of patients reporting visits (%)
Self-reported outpatient visits	
Within 26 weeks prior to initiation	2 (1.1)
Within 26 weeks prior to EOS or discontinuation	1 (0.5)
Self-reported emergency room visits	
Within 26 weeks prior to initiation	1 (0.5)
Within 26 weeks prior to EOS or discontinuation	0
Self-reported inpatient hospitalizations	
Within 26 weeks prior to initiation	0
Within 26 weeks prior to EOS or discontinuation	0
Self-reported episodes requiring assistance from an ambulance	
Within 26 weeks prior to initiation	1 (0.5)
Within 26 weeks prior to EOS or discontinuation	0
Self-reported episodes required administration of glucagon	
Within 26 weeks prior to initiation	0
Within 26 weeks prior to EOS or discontinuation	0
Self-reported workdays missed	
Within 26 weeks prior to initiation	0
Within 26 weeks prior to EOS or discontinuation	0

EOS: end of study; IDegAsp: insulin degludec/insulin aspart; HRU: healthcare resource utilization; n: number of participants contributing to the analysis; SD: standard deviation.

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ORIGINAL ARTICLE

Intentional Hyperglycemia at work, Glycemic Control, Work-related Diabetes Distress and Work Ability among Workers with Diabetes

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Abstract

Background. Work life of individuals with diabetes differs from that of those without diabetes. Work may interfere with diabetes self-management tasks, resulting in intentional hyperglycemia at work (IHW) and poor glycemic control. Diabetes affects work productivity due to work-related diabetes distress (WRDD) and impaired work ability (WA).

Objectives. To estimate the prevalence and identify the predictors of always high, poor/very poor glycemic control, high WRDD and poor/moderate WA among workers with diabetes.

Methodology. A cross-sectional study was done at the Specialized Medical Hospital Mansoura University, which included 323 working patients with diabetes. They were subjected to personal interviews to collect socio-demographic data, occupational, diabetes and other pertinent medical histories. Questionnaires for measuring IHW, WRDD and WA were completed. Clinical and A1c data were obtained from their records.

Results. The prevalence of always high IHW, poor/very poor glycemic control, high WRDD and poor/moderate work ability was: 23.8%, 60.1%, 34.7% and 74.6%, respectively. The predictors of always high IHW were: 1) Below university education; 2) Treatment with insulin only or combined with oral drugs; and 3) High WRDD. The predictors of poor/very poor glycemic control were urban residence, always and almost high IHW. The predictors of high WRDD were mentally-requiring jobs or both mentally- and physically-requiring jobs, duration of diabetes greater than 14 years and treatment with insulin. The predictors of poor/moderate WA were 'high' WRDD, 'almost high' and 'high a few times' IHW ratings.

Conclusions. Most of the studied population suffered mainly from poor/very poor glycemic control and poor/moderate work ability, while a lower proportion had high WRDD. This highlighted the need for workplace modifications and interventions to help workers with diabetes control their diabetes, improve their work ability and reduce WRDD to increase productivity.

Key words: glycemic control, work ability, intentional hyperglycemia, work-related diabetes stress, work, diabetes

INTRODUCTION

Many members of the workforce suffer from Diabetes mellitus (DM) and its complications worldwide. Although the American Diabetes Association (ADA) recommends that any qualified person has the right to join any job whether they have diabetes or not, employers still prioritize health status among the qualifications. Solving this problem is achieved through personally assessing each employee and their ability to do the job requirements safely and effectively, regardless of diabetes status.[1] Work life of working individuals with diabetes differs from work life of the general population without diabetes as the daily burden of disease management negatively affects work opportunities and choices.[2] Work is also affected by factors influencing illness perceptions and self-care practices of workers and employees with diabetes.[3]

There is a reciprocal relationship between work and diabetes through work-related diabetes distress (WRDD), which is one of the psychosocial concerns reflecting how often working adults with diabetes are worried about the ability to do their jobs because of diabetes.[4] Similarly, diabetes may affect work ability.[5] Also, work may interfere with diabetes self-management tasks such as controlling the blood glucose at recommended levels to prevent or delay complications.[6]

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As efforts towards the investigation of the relationship between diabetes mellitus and work are few in Egypt, this study will highlight some of the effects of working with diabetes and how it affects workers' health and working ability.

OBJECTIVES

To estimate the prevalence and identify predictors of always high intentional hyperglycemia at work (IHW), poor/very poor glycemic control, high work-related diabetes distress and poor/moderate work ability.

Population and methods

This cross-sectional study was done in the Specialized Medical Hospital, Mansoura University, from April 2022 to February 2023.

Inclusion criteria

The study included adult outpatients with diabetes who were working during the study period and who agreed to participate in the study.

Exclusion criteria

Patients who worked for ≤1 year only and were only recently diagnosed with diabetes(for ≤1 year only) were excluded from the study. A pilot study was done on 65 patients (not included in the full-scale study) to test questionnaire applicability, tool reliability and estimate sample size.

Sample size

To estimate the prevalence of IHW in the target population, a minimum sample size of 318 patients was required. This calculation was based on the sample size formula for estimating a population proportion and was determined using the following information: 1) an anticipated prevalence rate of 21.5%, derived from a previous pilot study; 2) a confidence level of 97%; and 3) a precision level of 5%. The sample size was computed using the Open Epi website.[7]

Data collection

Sampling method

All outpatients with diabetes who accepted to participate, fulfilled the inclusion criteria of having diabetes for more than one year and were also working were asked to complete a specially designed questionnaire covering:

A. ***The socio-demographic data*** (age, sex, marital status, educational level and residence), occupational history (job title, job duration, working hours/day, job requirement, shift work) and diabetes history (type, duration, treatment)

B. Tools:

Intentional hyperglycemia at work (IHW)

Intentional hyperglycemia at work refers to the intention of the worker/employee with diabetes to maintain a high blood glucose level at work. Intentional hyperglycemia was assessed using a single item. Respondents were asked to rate how often they intended to maintain high blood glucose levels at work, using a 5-point Likert scale from "never" to "always." This measure has been validated in a previous survey among the adult working population with diabetes[4] and through qualitative research.[3]

Glycemic control

The last reported glycosylated hemoglobin (A1c) level was abstracted from the medical record and classified into good glycemic control = A1c <7%, fair control = A1c 7–8% and poor control = A1c > 8.0%. An A1c cut-off value of ≥9% was used to represent very poor control.[8]

Work-related diabetes distress (WRDD)

The respondents were asked to rate how often they worried about their ability to do their job due to their diabetes and how often they became exhausted by the need to reconcile their work with their diabetes. On a scale of 1–3 (never, sometimes, often), a sum score was calculated. The median was taken as an arbitrary cut-off point. Higher scores indicated higher WRDD.[4]

For IHW and WRDD, **the adverbs of how often an action was done are operationally defined as: always** (doing an action 100% of the time, all the time with no fail); **always high** (75-99% most of the time it happens); **almost high** (50-74% indicating a habit that is usual or common); **high a few times** (does action 25-49% of the time; **rarely high** (1-25% of the time); and **never** (the opposite of always; doing action 0% of the time or not at all).

Work Ability (WA)

Work ability refers to the ability to function well at work or achieve expected work goals, measured by the Work Ability Index (WAI), a self-report assessment tool comprised of seven items.[9,10]

Item 2 denotes work ability in relation to the demands of the job. For physically demanding work, the work ability score is multiplied by 1.5, and the work ability score for mentally demanding work is multiplied by 0.5. For mentally demanding work, the work ability score for the physical demands of the job is multiplied by 0.5, and the work ability score for the mental demands of the job is multiplied by 1.5. For work that is both physically and mentally demanding, the work ability score remains unchanged. For item 3 (number of current diseases diagnosed by a physician), scoring is as follows: 5 or more diseases = 1 point, 4 diseases =2 points, 3 diseases = 3 points, 2 diseases = 4 points, 1 disease = 5 points, no disease = 7 points (only diseases diagnosed by a physician are counted). For item 7 (mental resources), it is divided into three questions that are added together, and

the sum is modified as follows: Sum of 0–3 = 1 point, 4–6 = 2 points, 7–9 = 3 points and 10–12 = 4 points. The WAI score is the sum of the seven scores (range 7–49). WAI scores are interpreted such that work ability is poor (7–27), moderate (28–36), good (37–43) or excellent (44–49).[11,12]

The English versions of the three tools (IHW, WRDD and WAI) were forward translated into the Arabic language by two translators producing the initial version. This initial version was backtranslated into English by another two bilingual translators who were unaware of the original version. A few discrepancies were agreed upon by consensus to get the final Arabic version.

The content validity of the WRDD and WAI Arabic was tested by a jury of 10 experts (professors in Public Health and Endocrinology). The content validity index (CVI) per item for WRDD ranged from 0.7 to 1.0 for both relevance and clarity, while CVI per expert ranged from 0.6 to 1.0 for both relevance and clarity. The content validity index (CVI) per item for WAI ranged from 0.8 to 1.0 for both relevance and clarity, while CVI per expert ranged from 0.63 to 1.0 for both relevance and clarity.

To test the reliability of the WRDD and WAI Arabic, the final versions were tested in the pilot study, and Cronbach's α reliability coefficients were 0.766 and 0.751, respectively. Data collection included interviews with patients and the collection of clinical data from their records.

Data analysis

Frequencies and percentages were computed to describe the responses to categorical variables measured in the study. To answer the study objectives, the point and 95% confidence interval (CI) estimates of the prevalence of IHW were computed. Furthermore, logistic regression analyses were performed to identify the predictors of the IHW, poor glycemic control, WRDD and poor WA among the patients. The chi-square test and simple logistic regression were performed for the bivariate analysis to ascertain which variables should be included in the multiple logistic regression model. After this, variable selection was performed using the forward selection method. The probability of entering the model was set at 0.05. Likewise, the significance level for all the hypothesis tests performed was 0.05. The crude odds ratio (COR) and adjusted odds ratio (AOR) were reported along with their corresponding 95% CIs. No imputation was performed in this study. All the analyses were conducted using SPSS software (version 17.0 for Windows; SPSS Inc., Chicago, IL, USA).

Ethical considerations

Informed verbal consent was obtained from each participant sharing in the study after assuring confidentiality. The data collection and examination were done by the investigator in privacy. The study proposal was approved by the Institutional Research Board (IRB): R.22.04.1673.

Table 1. Socio-demographic, occupational and diabetes characteristics among the study population

Characteristics	Total No. (%)
Overall	323 (100.0)
Age (years)	
20-55	214 (66.3)
≥56	109 (33.7)
Mean ± SD	49.9 ± 9.7
Sex	
Male	216 (66.9)
Female	107 (33.1)
Marital status	
Married	277 (85.8)
[§]Unmarried	46 (14.2)
Residence	
Urban	161 (49.9)
Rural	162 (50.1)
Education	
Below university	225 (69.7)
University and above	98 (30.3)
Job requirement	
Physical	29 (8.9)
Mental	31 (9.6)
Both	263 (81.5)
Working years	
≤10	54 (16.7)
>10	269 (83.3)
Mean ± SD	24.8 ± 12.3
Working hours	
≤8	227 (70.3)
>8	96 (29.7)
Mean ± SD	8.3 ± 2.4
Night shift	
Yes	69 (21.4)
No	254 (78.6)
Diabetes Mellitus	
Type 1	116 (35.9)
Type 2	207 (64.1)
Diabetes duration (years)	
≤14	165 (50.1)
>14	158 (49.9)
Mean ± SD	14.1 ± 8.5
Family history	
Positive	191 (59.1)
Negative	132 (40.9)
Treatment	
Oral	94 (29.1)
Insulin	174 (53.9)
Both	55 (17.0)

[§]Unmarried: single or widow or divorced

RESULTS

Table 1 shows that most of the participants were males, ages 20 to 55 years, married and educated below university (66.9%,66.3%, 85.8% and 69.7%, respectively). Most of the participants worked ≤8 hours per day for more than 10 years, without night shifts in jobs requiring both physical and mental capacities (70.3%,83.3%,78.6% and 81.5%, respectively). The majority of the patients had type 2 diabetes, and about half of the participants had diabetes for ≤14 years, had a positive family history of diabetes and received insulin alone for treatment at 50.1%,59.1% and 53.9%, respectively.

Table 2 shows that the majority of patients had poor glycemic control defined as an HbA1c greater than 8% (60.1%), almost and always with IHW (52%), had low WRDD (65%), and poor/moderate work ability (74.6%).

Table 2. Distribution of IHW, glycemic control, WRDD and work ability among the study population

Outcome	323 (100) N (%)	(95% CI) of proportion
IHW		
always high	77 (23.8)	(19.2-28.5)
almost high	92 (28.5)	(23.6-33.4)
high few times	92 (28.5)	(23.6-33.4)
rarely high	38 (11.8)	(11.8-15.3)
never high	24 (7.4)	(4.6-10.3)
Glycemic Control		
good (A1c <7%)	48 (14.9)	(11.0-18.7)
fair (A1c 7–8%)	81 (25.1)	(20.4-29.8)
poor, very poor (A1c >8.0%)	194 (60.1)	(54.5-65.4)
WRDD		
Low (1-4)	211 (65.3)	(60.1-70.5)
High (5-6)	112 (34.7)	(29.5-39.9)
WA		
poor/moderate (7-36)	241 (74.6)	(69.5-79.3)
good/excellent (37-49)	82 (25.4)	(20.6-30.1)

IHW: intentional hyperglycemia at work; WRDD: work-related diabetes distress; WA: work ability

Table 3 shows that the overall prevalence of always high IHW was 23.8% (95% CI [19.2,28.5]). The significant independent predictors of always high IHW among participants were: 1) being educated below university (AOR: 2.1,95% CI [1.1, 3.9]), 2) treated with insulin alone or combined with oral drugs (AOR: 3.1,95% CI [1.4, 6.8] and AOR: 4.2,95% CI [1.7-10.8], respectively) and high WRDD (AOR: 2.5,95% CI [1.5, 4.5]).

Table 4 shows that the overall prevalence of poor glycemic control was 60.1% (95% CI [54.5-65.4]). The significant independent predictors of poor/very poor glycemic control among participants were the following: living in an urban area (AOR: 1.8,95% CI [1.1, 2.9]), always high IHW (AOR:5.6,95% CI [2.0, 15.2]) and almost high IHW (AOR:3.8,95% CI [1.4, 10.0]).

Table 5 shows that the overall prevalence of high WRDD was 34.7% (95% CI [29.5,39.9]). The significant independent

Table 3. Factors associated with and independent predictors of the always high IHW

Characteristics	Total No.	IHW Always high No. (%)	Test of significance *P* value	COR (95% CI)	Regression analysis	
Overall	323	77 (23.8)		(19.2-28.5)	*P*	AOR (95% CI)
Age (years)			*P* = 0.9			
20-55 (r)	214	51 (23.8)		Referent		
≥56	109	26 (23.9)		1.1 (0.6-1.7)		
Sex			*P* = 0.3			
Male (r)	216	48 (22.2)		Referent		
Female	107	29 (27.1)		1.3 (0.8-2.2)		
Marital status			*P* = 0.7	1		
Married (r)	277	65 (23.5)		1.2 (0.6-2.4)		
§Unmarried	46	12 (26.1)				
Residence			*P* = 0.3			
Urban (r)	161	34 (21.1)		Referent		
Rural	162	43 (26.5)		1.4 (0.8-2.3)		
Education			*P* = 0.02		0.02	
Below University	225	62 (24.3)		2.1 (1.13-3.9)		2.1 (1.1-3.9)
University and above (r)	98	15 (15.3)		Referent		Referent
Job requirement						
Physical (r)	29	5 (17.2)	*P* = 0.6	Referent		
Mental	31	7 (22.6)	*P* = 0.4	1.4 (0.4-5.0)		
Both	263	65 (24.7)		1.6 (0.6-4.3)		
Working years			*P* = 0.5			
≤10 (r)	54	11 (20.4)		Referent		
>10	269	66 (24.5)		1.3 (0.6-2.6)		
Working hours			*P* = 0.6			
≤8	227	56 (24.7)		1.2 (0.7-2.1)		
>8 (r)	96	21 (21.9)		Referent		
Night shift			*P* = 0.9			
Yes	69	17 (24.6)		1.1 (0.6-1.9)		
No (r)	254	60 (23.6)		Referent		
Diabetes Mellitus			*P* = 0.2			
Type 1 (r)	116	23 (19.8)		Referent		
Type 2	207	54 (26.1)		1.4 (0.8-2.4)		
Diabetes duration (years)			*P* = 0.06			
≤14 (r)	165	32 (13.9)		Referent		
>14	158	45 (28.5)		1.7 (1.0-2.8)		
Family history			*P* = 0.7			
Positive	191	47 (24.6)		1.1 (0.7-1.9)		
Negative (r)	132	30 (22.7)		Referent		
Treatment						
Oral (r)	94	9 (9.6)	*P* ≤0.001	Referent	0.004	Referent
Insulin	174	52 (29.9)	*P* = 0.002	4.0 (1.9-8.6)	0.002	3.1 (1.4-6.8)
Both	55	16 (29.1)		3.9 (1.6-9.5)		4.2 (1.7-10.8)
WRDD			*P* ≤0.001			
High	112	42 (37.5)		3.0 (1.8-5.1)	0.001	2.5 (1.5-4.5)
Low (r)	211	35 (16.6)		Referent		Referent
Median (min-max)	4 (2-6)					

§Unmarried: single or widow or divorced, (r) constant
IHW: intentional hyperglycemia at work; WRDD: work-related diabetes distress

predictors of high WRDD among participants were mental job requirement alone (AOR: 4.0, 95% CI [1.1, 15.2]), or combined mental and physical requirement (AOR: 3.2, 95% CI [1.1-9.8]), duration of diabetes >14 years (AOR: 1.8, 95% CI [1.05, 2.9]) and treatment with insulin (AOR: 2.0, 95% CI [1.02, 3.7])

Table 6 shows that the overall prevalence of poor/moderate work ability was 74.6% (95% CI [69.5, 79.3]). The significant independent predictors of poor/moderate WA among participants were high WRDD (AOR:13.2,95% CI [4.6-37.8]), IHW always high (AOR(95%CI): 5.8(2.1-15.8)], almost high [AOR (95%CI): 4.5(1.8-11.7)] and high few times {AOR(95%CI): 3.2(1.3-7.9)].

DISCUSSION

The present study revealed that the prevalence of patients who intended to keep their blood glucose level at work (IHW) always high was 23.8%, which can be explained by the fear of hypoglycemia at the workplace or "strategic non-compliance."[13] However, lower rates (1.6% and 4.6%) were reported among workers with diabetes in Denmark[14] and Finland,[4] respectively. The discrepancy between the prevalence in the current study and the previous ones could be due to the difference in health knowledge, lifestyle and income between a developing country in Africa and developed Europe.

Table 4. Factors associated with and independent predictors of the poor/very poor glycemic control

Characteristics	Total No.	Poor/very poor glycemic control, No. (%)	Test of significance *P* value	COR (95% CI)	Regression analysis	
Overall	323	194 (60.1)		(54.5-65.4)	*P*	AOR (95% CI)
Age (years)			*P* = 0.2			
20-55	214	123 (57.5)		1.3 (0.9-2.2)		
≥56 (r)	109	71 (65.1)		Referent		
Sex			*P* = 0.2			
Male	216	135 (62.5)		1.4 (0.8-2.2)		
Female (r)	107	59 (55.1)		Referent		
Marital status			*P* = 0.7			
Married (r)	277	165 (59.6)		Referent		
§Unmarried	46	29 (63.0)		1.2 (0.6-2.2)		
Residence			*P* ≤0.001		0.02	
Urban	161	107 (66.5)		1.7 (1.1-2.7)		1.8 (1.1-2.9)
Rural (r)	162	87 (53.7)		Referent		Referent
Education			*P* = 0.3			
Below University	225	139 (61.8)		1.2 (0.8-2.0)		
University and above (r)	98	55 (56.1)		Referent		
Job requirement						
Physical	29	18 (62.1)	*P* = 0.4	1.5 (0.5-4.3)		
Mental (r)	31	16 (51.6)	*P* = 0.3	Referent		
Both	263	160 (60.8)		1.5 (0.7-3.1)		
Working years			*P* = 0.05			
≤10 years	54	39 (72.2)		1.9 (1.01-3.6)		
>10 years (r)	269	155 (57.6)		Referent		
Working hours			*P* = 0.7			
≤8 (r)	227	135 (59.4)		Referent		
>8	96	59 (61.5)		0.9 (0.5-1.5)		
Night shift			*P* = 0.7			
Yes	69	43 (62.3)		1.1 (0.7-1.8)		
No (r)	254	151 (59.4)		Referent		
Diabetes Mellitus			*P* = 0.9			
Type 1	116	70 (60.3)		1.02 (0.6-1.6)		
Type 2 (r)	207	124 (59.9)		Referent		
Diabetes duration (years)			*P* = 0.001			
≤14 (r)	165	84 (50.9)		Referent		
>14	158	110 (69.6)		2.2 (1.4-3.5)		
Family history			*P* = 0.1			
Positive (r)	191	108 (56.5)		Referent		
Negative	132	86 (65.2)		1.4 (0.9-2.3)		
Treatment						
Oral (r)	94	44 (46.8)	*P* = 0.002	Referent		
Insulin	174	112 (64.4)	*P* = 0.008	2.1 (1.3-3.4)		
Both	55	38 (69.1)		2.5 (1.3-5.1)		
WRDD			*P* = 0.1			
High	112	74 (66.1)		1.5 (0.9-2.4)		
Low (r)	211	120 (56.9)		Referent		
Median (min-max)	4 (2-6)					
IHW						
Always high	77	57 (74.0)	*P* ≤0.001	5.7 (2.1-15.3)	0.001	5.6 (2.0-15.2)
Almost high	92	62 (67.4)		4.1 (1.5-10.7)	0.007	3.8 (1.4-10.0)
High few times	92	47 (51.1)	*P* = 0.002	2 (0.8-5.3)	0.1	2.1 (0.8-5.3)
Rarely high	38	20 (52.6)	*P* = 0.09	2.2 (0.8-6.4)	0.1	2.2 (0.8-6.4)
Never high (r)	24	8 (33.3)	*P* = 0.1	Referent		Referent

§Unmarried: single or widow or divorced, (r) constant, ≈ A1c>8.0%
IHW: intentional hyperglycemia at work; WRDD: work-related diabetes distress

Table 5. Factors associated with and independent predictors of the high WRDD

Characteristics	Total No.	High WRDD No. (%)	Test of significance *P* value	COR (95% CI)	Regression analysis	
Overall	323	112 (34.7)		(29.5-39.9)	***P***	AOR (95% CI)
Age (years)			*P* = 0.9			
20-55(r)	214	74 (34.6)		Referent		
≥56	109	38 (34.8)		1.01 (0.6-1.6)		
Sex			*P* = 0.3			
Male (r)	216	71 (32.9)		Referent		
Female	107	41 (38.3)		1.3 (0.8-2.1)		
Marital status			*P* = 0.2			
Married	277	100 (36.1)		1.6 (0.8-3.2)		
§Unmarried (r)	46	12 (26.1)		Referent		
Residence			*P* = 0.1			
Urban (r)	161	49 (30.4)		Referent		
Rural	162	63 (38.9)		1.5 (0.9-2.3)		
Education			*P* = 0.07			
Below University	225	85 (37.8)		1.6 (0.9-2.7)		
University and above (r)	98	27 (27.6)		Referent		
Job requirement						
Physical (r)	29	4 (13.8)	*P* = 0.03	Referent		Referent
Mental	31	12 (38.7)	*P* = 0.01	3.9 (1.1-14.2)	0.04	4.0 (1.1-15.2)
Both	263	96 (36.5)		3.6 (1.2-10.6)	0.04	3.2 (1.1-9.8)
Working years			*P* = 0.07			
≤10 years (r)	54	13 (20.4)		Referent		
>10 years	269	99 (36.8)		1.8 (0.9-3.6)		
Working hours			*P* = 0.3			
≤8 (r)	227	75 (33.0)		Referent		
>8	96	37 (38.5)		1.3 (0.8-2.1)		
Night shift			*P* = 0.9			
Yes	69	24 (34.8)		1.1 (0.6-1.7)		
No (r)	254	88 (34.6)		Referent		
Diabetes Mellitus			*P* = 0.001			
Type 1 (r)	116	27 (23.3)		Referent		
Type 2	207	85 (41.1)		2.3 (1.4-3.8)		
Diabetes duration (years)			*P* ≤0.001		0.03	
≤14 (r)	165	43 (26.1)		Referent		Referent
>14	158	69 (43.7)		2.2 (1.4-3.5)		1.8 (1.05-2.9)
Family history			*P* = 0.7			
Positive	191	68 (35.6)		1.1 (0.7-1.8)		
Negative (r)	132	44 (33.3)		Referent		
Treatment					0.04	
Oral (r)	94	20 (21.3)	*P* ≤0.001	Referent		Referent
Insulin	174	77 (44.3)	*P* = 0.4	2.9 (1.6-5.2)		2.0 (1.02-3.7)
Both	55	15 (27.3)		1.4 (0.6-3.0)		-

§Unmarried: single or widow or divorced, (r) constant
IHW: intentional hyperglycemia at work; WRDD: work-related diabetes distress

The current study (Table 3) found that those patients with education less than the university level had a higher risk of always hyperglycemia at work, which is associated with worse glycemic control.[5] This finding could result from insufficient health knowledge as a lower educational level hinders the patient's ability to care for their diabetes. This result was supported by a study in the US, which detected that the patients with diabetes with low educational levels had a negative effect on their blood glucose level.[15]

Also, the present data showed that working adults treated with insulin only or combined with oral drugs have a higher risk of always having high blood glucose at work and this can be related to skipping insulin doses because of the unavailability of either a private, clean place to do the injection or an insulated carrier or fridge to preserve it.[16] Similarly, in Ethiopia, patients treated with insulin only or combined with oral drugs were at greater risk of poor glycemic control than those on oral anti-diabetic drugs alone.[17]

Current data also found that high WRDD increased the risk of hyperglycemia at work. Having high WRDD is a source of psychological and emotional stress that may contribute to hyperglycemia among patients with diabetes, and this was in accordance with the study finding that high WRDD resulted in more frequent IHW.[5] Similarly, the association between WRDD and IHW was detected in the Finnish study.[4]

The prevalence of poor/very poor glycemic control among the workers with diabetes in the current study was 60.1%, and this may be due to either non-compliance with medications or poor diet. Studies in Ethiopia[18] and Amman, Jordan[19] have shown the prevalence of poor/very poor glycemic control was 61.92% and 65.1%, respectively. In contrast, studies in Mumbai, India, found a higher prevalence of poor/very poor glycemic control (91.8%) due to a lack of self-care management behaviors in addition to a long duration of diabetes.[20] The current study revealed that the risk of poor/very poor glycemic control was higher among urban residents and can be attributed to unhealthy

lifestyles and diets. This finding is consistent with another study in Saudi Arabia.[21] However, studies in Ethiopia reported that the greater risk of poor glycemic control was among patients living in rural areas than those living in urban Ethiopia.[22]

The present work reflected a higher risk of poor/very poor glycemic control among those with always or almost high IHW. In agreement with this finding, studies proved the association between poor/very poor glycemic control and IHW among Finnish workers.[4] However, a direct relationship between glycemic control and IHW was not established. Instead, an indirect effect through an intermediate pathway through the effect of work-related factors on glycemic control was observed.[5]

The current study showed that the overall prevalence of high WRDD was 34.7%, which is lower than that detected among Finnish workers with diabetes (70%).[4] In this present work, the risk of high WRDD increased among those exposed to mental job requirements alone or combined with physical requirements which were in contrast to studies proving that mental work protects from WRDD.[4]

Table 6. Factors associated with and independent predictors of the poor/moderate WA

Characteristics	Total No.	Poor/ moderate WA¥	Test of significance *P* value	COR (95% CI)	Regression analysis	
Overall	323	241 (74.6)		(69.5-79.3)	*P*	AOR (95% CI)
Age (years)			*P* = 0.2			
20-55 (r)	214	155 (72.4)		Referent		
≥56	109	86 (78.9)		1.4 (0.8-2.5)		
Sex			*P* = 0.9			
Male (r)	216	161 (74.5)		Referent		
Female	107	80 (74.8)		1.01 (0.6-1.7)		
Marital status			*P* = 0.2			
Married	277	210 (75.8)		1.5 (0.8-3)		
§Unmarried (r)	46	31 (67.4)		Referent		
Residence			*P* = 0.2			
Urban (r)	161	115 (71.4)		Referent		
Rural	162	126 (77.8)		1.4 (0.8-2.3)		
Education			*P* = 0.04			
Below University	225	175 (77.8)		1.7 (1.003-2.9)		
University and above (r)	98	66 (67.3)		Referent		
Job requirement						
Physical (r)	29	18 (62.1)	*P* = 0.06	Referent		
Mental	31	26 (83.9)	*P* = 0.1	3.2 (0.9-10.7)		
Both	263	197 (74.9)		1.8 (0.8-4)		
Working years			*P* = 0.4			
≤10 (r)	54	38 (70.4)		Referent		
>10	269	203 (75.5)		1.3 (0.7-2.4)		
Working hours						
≤8 (r)	227	168 (74.0)	χ^2 = 0.15	Referent		
>8	96	73 (76.0)	*P* = 0.7	1.1 (0.6-1.9)		
Night shift			*P* = 0.9			
Yes	69	51 (73.9)		1.1 (0.5- 1.9)		
No (r)	254	190 (74.8)		Referent		
Diabetes Mellitus			*P* = 0.03			
Type 1 (r)	116	76 (65.5)		Referent		
Type 2	207	165 (79.7)		1.8 (1.1-2.9)		
Diabetes duration (years)			*P* = 0.07			
≤14 (r)	165	116 (70.3)		Referent		
>14	158	125 (79.1)		1.6 (0.9-2.6)		
Family history			*P* = 0.3			
Positive (r)	191	139 (72.8)		Referent		
Negative	132	102 (77.3)		1.3 (0.8-2.1)		
Treatment						
Oral	94	66 (70.2)	*P* = 0.7	1.1 (0.6-2.3)		
Insulin	174	138 (79.3)	*P* = 0.1	1.9 (0.9-3.7)		
Both (r)	55	37 (67.3)		Referent		
Glycemic control			*P* = 0.6			
Poor/very poor	194	147 (75.8)		1.2 (0.7-1.9)		
Good/fair (r)	129	94 (72.8)		Referent		
WRDD			*P* ≤0.001		≤0.001	
High	112	108 (96.4)		15.8 (5.6-44.6)		13.2 (4.6-37.8)
Low (r)	211	133 (63.0)		Referent		Referent
Median (min-max)	4 (2-6)					
IHW						
Always high	77	64 (83.1)	*P* = 0.0003	5.8 (2.1-15.8)	0.001	5.8 (2.1-15.8)
Almost high	92	73 (79.3)	*P* = 0.001	4.5 (1.7-11.7)	0.002	4.5 (1.8-11.7)
High few times	92	67 (72.8)	*P* = 0.01	3.2 (1.3-7.9)	0.02	3.2 (1.3-7.9)
Rarely high	38	26 (68.4)	*P* = 0.08	2.6 (0.9-7.3)	0.08	2.6 (0.9-7.3)
Never high (r)	24	11 (45.8)		Referent		Referent

§Unmarried: single or widow or divorced, (r) constant
¥Poor/ moderate WA: WAI score = 7-36
IHW: intentional hyperglycemia at work; WRDD: work-related diabetes distress; WA: work ability

Also, this study found that those having diabetes for >14 years duration or being treated with insulin had an increased risk of high WRDD. In agreement with our results, studies in India reported that insulin use increased the risk of WRDD.[23] Similarly, studies in Ethiopia found that both the long duration of diabetes >10 years and insulin therapy increased the risk of poor glycemic control,[24] causing higher WRDD.[4]

The present work found that 74.6% of the participants had poor/moderate work ability, which could be attributed to high blood glucose related to poor glycemic control and intentional hyperglycemia at work. Our finding was consistent with a study in Iran, which detected that 75% of the participants had poor/moderate work ability.[25] Similarly, other studies supported our findings.[26,27] The present study found that the predictors of poor/moderate work ability were as follows: high WRDD, always high, almost high or high a few times IHW. These findings were in agreement with a study in Denmark.[5]

Limitations

Since the sample size for this study was computed primarily to estimate the prevalence of IHW among patients, the power of the statistical tests was ascertained to determine possible issues with the external validity of the modeling results. Consequently, post-hoc power analysis showed that most predictors included in the final models had adequate power of 0.80 or higher, except the hypothesis test involving the regression coefficient for education in the IHW model, which only had a power of 0.54. Nevertheless, the CIs for this variable indicate that low education likely increases the odds of having a negative outcome.

CONCLUSIONS

The results from our study demonstrated that most of the studied population had poor/very poor glycemic control and poor/moderate work ability, with a lower proportion having high WRDD. This highlighted the need for workplace modifications and interventions to help workers with diabetes achieve good glucose control, improve their work ability and reduce WRDD to increase production.

Recommendations

Health education must be regularly offered to all patients attending the diabetes clinic, focusing on work life with diabetes. Encouraging communication with the occupational health personnel for minor work modifications to reduce WRDD and to devise means at work to allow space and time for small meals, self-monitoring of blood glucose and/or receiving treatment. Further multicenter studies are recommended to express the magnitude of the problem on a larger scale.

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

CRediT Author Statement
SSE: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft preparation, Writing - review and editing, Visualization, Supervision, Project administration, Funding acquisition; **ABEG:** Conceptualization, Methodology, Software, Validation, Formal analysis, Data curation, Writing - review and editing; **MRGB:** Conceptualization, Resources, Data curation; **AME:** Conceptualization, Investigation, Resources, Data curation

Data Availability Statement
The datasets generated and/or analyzed during the current study are not publicly available due to the privacy of patients attending this hospital but are available from the corresponding author upon reasonable request.

Author Disclosure
The authors declared no conflict of interest.

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CASE REPORT

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R243W Mutation in Thyroid Hormone Resistance Syndrome Beta: A Case Report

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Abstract

A three-year-old girl with a history of recurrent tonsillitis was investigated for failure to thrive and global developmental delay. Clinically, she had a triangular face with low-set ears and intermittent tachycardia. She had growth failure with her weight under the third centile while her height was within normal limits. Other systemic examinations were unremarkable. The presence of an elevated free T4 (FT4) with an inappropriately high thyroid stimulating hormone (TSH) in this patient raised the clinical suspicion of Thyroid Hormone Resistance Syndrome. DNA sequencing confirmed the diagnosis, which showed R243W gene mutation in Thyroid Hormone Receptor-Beta1 (THRB1).

Key words: thyroid hormone receptor, thyroid hormone resistance, goiter

INTRODUCTION

Thyroid Hormone Resistance Syndrome (THR) is a rare condition affecting 1 in 40,000 live births. It is secondary to defects in either of the two thyroid hormone receptors-alpha and beta, leading to tissue unresponsiveness to circulating thyroid hormone.[1] The disorder might be mistaken for congenital hypothyroidism, Graves' disease or other forms of autoimmune thyroiditis. For these reasons, patients will be unnecessarily maintained on thyroxine replacement for hypothyroidism, while some may be subjected to thyroidectomy without considering the primary diagnosis of THR. Careful interpretation of thyroid function tests (TFT) and referral to a paediatric endocrinologist are essential to prevent misdiagnosis. The hypothalamic-pituitary-thyroid axis regulates thyroid hormone. Thyroid hormone exerts negative feedback on the anterior pituitary gland and hypothalamus to suppress TSH and thyroid-releasing hormone (TRH). Typically, patients with hypothyroidism or hyperthyroidism would present with either a low or high FT4 and a high or suppressed TSH, respectively. On the other hand, patients with THR would have a loss of this normal feedback regulation and would present with atypical TFTs characterized by an elevation of both FT4 and TSH. We present a case of a three-year-old girl who was diagnosed with THR.

CASE

A three-year-old girl who had recurrent admissions for acute tonsillitis was investigated due to failure to thrive, global developmental delay and intermittent tachycardia. She was born term, SGA, via spontaneous vaginal delivery, with a birth weight of 1.87 kg. Her neonatal period was uneventful. She had three hospitalizations for acute tonsillitis and was diagnosed with global developmental delay. She could walk independently at age two and could only speak in phrases at three years of age. She could put on socks and shoes but could not feed herself. Her paternal grandfather had goiter and underwent surgery, but the exact diagnosis was not known. He passed away at the age of 50 due to unknown reasons. Physically, she had a triangular face and low-set ears. No goitre or skeletal dysplasia was noted (Figure 1). Although her weight was persistently under the 3rd centile from birth until her current presentation, her height was consistently at the 10th centile. Her baseline heart rate was 110 beats per minute. Other systemic examinations were unremarkable.

A thyroid function test (TFT) was done to investigate the etiology of her failure to thrive and tachycardia. Results showed high FT4 and TSH. Table 1 summarizes her serial TFTs. She had persistently elevated TSH, FT4 and FT3. Other blood investigations are shown in Table 2.

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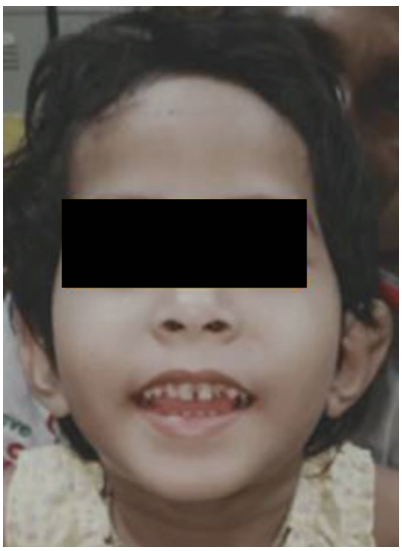

Figure 1. Soft dysmorphism with low-set ears.

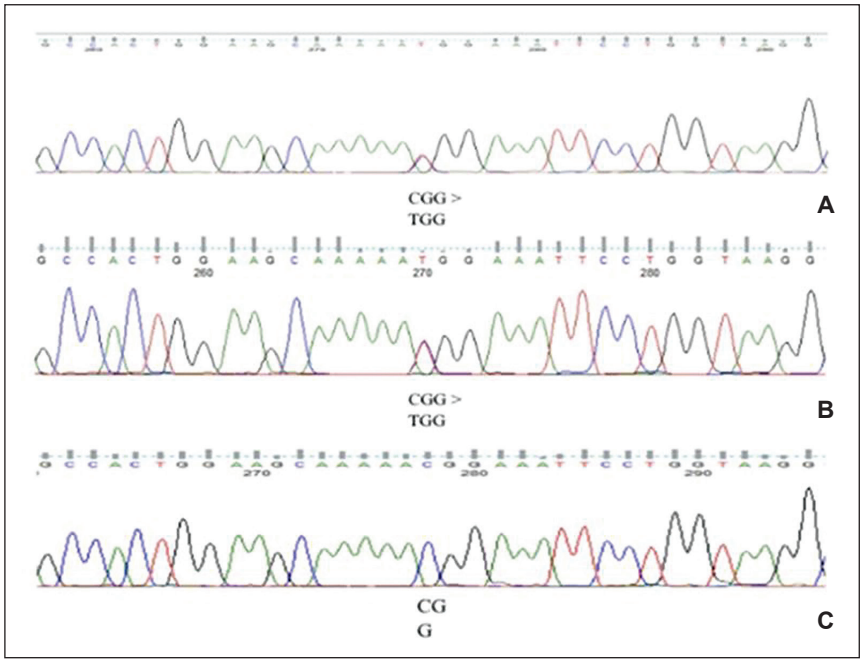

Figure 2. Sanger sequencing results for THRB1 gene. **(A)** and **(B)** represent the patient and her father respectively; **(C)** is the result of the mother.

Table 1. Serial Thyroid Function Test

Age	3 year 5 month old	3 year old 6 month old	5 year 10 month old	7 year old	7 year 11 month old
TSH (mIU/L)	26.48	18.41	4.74	5.97	6.88
FT4 (pmol/L)	42.0	55.74	50.67	51.23	47.10
FT3			16.02		
Ratio FT4/FT3			3.16		

Normal range: TSH: 0.5-4 mIU/L; FT4: 10-24 pmol/L; FT3: 3.1-6.8 pmol/L; FT4/FT3 ratio: 2.2-3.7

Autoimmune testing such as thyroid-stimulating hormone receptor (TRAb), anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG) antibodies were negative. MRI of the pituitary gland and skeletal survey were normal. Ultrasound of the thyroid gland showed goiter, with the right thyroid gland measuring 0.8 x 1.4 x 3 cm and the left thyroid gland measuring 0.9 x 1.4 x 3.3 cm with generalized increased vascularity within both glands. Due to the expression of thyroid receptors in the muscle and liver, a lipid profile and creatinine kinase were done, which showed normal results.

Family screening with thyroid function tests was done as those who carry the mutations may be asymptomatic and do not require treatment. For this family, the patient is the third of four siblings. Thyroid disease ran in the family as both her paternal grandfather and father showed similar TFT patterns of an elevated FT4 and a non-suppressed TSH. His FT4 was 36.27 pmol/L while TSH was 1.76 mIU/L. DNA sequencing showed that the patient and her father have the same mutation (R243W mutation) in the thyroid hormone receptor-beta gene, as reported in a previous study.[2] On the other hand, her mother was not affected (Figure 2). Other siblings were not screened as they were asymptomatic.

Table 2. Investigations

Calcium (mmol/L)	2.4
Phosphate (mmol/L)	1.78
Hemoglobin (g/dL)	13.7
Cholesterol (mmol/L)	5.3
Triglyceride (mmol/L)	0.44
Creatinine kinase (U/L)	143
TSH receptor antibody	negative
ATG (kIU/L)	14.03 (<115)
TPO (KIU/L)	10.12 (<34)

ATG: Antithyroglobulin antibody
TPO: Thyroid Peroxidase antibody
TSH: Thyroid Stimulating Hormone

DISCUSSION

THR syndrome can be classified into three types: pituitary THR, peripheral THR and generalized THR.[3] In pituitary THR, the pituitary gland cannot sense the circulating thyroid hormone, thus leading to increased secretion of TSH and, subsequently, elevated thyroid hormone.[4] Peripheral THR is caused by tissue insensitivity to thyroid hormone without pituitary gland involvement. They present with normal thyroid hormone and TSH levels but may manifest with symptoms of hypothyroidism. In generalized THR, peripheral tissues and the pituitary gland are resistant to thyroid hormone due to receptor defects causing both elevated TSH and thyroid hormone.[3] This patient is consistent with pituitary THR as both TSH and FT4 were elevated with thyrotoxic symptoms.

The thyroid receptor (TR) consists of alpha-1, alpha-2, beta-1 and beta-2. TR alpha-1 is expressed specifically in the heart and muscles, while little is known about alpha-2 receptors. TR beta-1 is expressed in the brain, liver and kidneys, while beta-2 is expressed in the hypothalamus and pituitary gland.[5] Different TH receptor tissue expressions result in different clinical manifestations. The majority of THR-beta (THRB) patients have goitre, palpitations, developmental delay, short stature and repeated infections.[6] Dysmorphisms such as bird-like facies and pigeon chest were reported previously but was absent in our patient.[7] Our patient had clinical clues consistent with THRB, such as the failure to thrive, developmental delay, intermittent tachycardia and repeated infection/tonsillitis. Patients with THR-alpha may exhibit dysmorphism, skeletal dysplasia, constipation and intellectual deficits that may mimic features of congenital hypothyroidism.

Concomitant FT4 and FT3 elevation with a non-suppressed TSH are typical findings in THR-beta syndrome. Increased deiodinase activity in THRB results in a high FT4/FT3 ratio.[8] However, THR-alpha syndrome can be differentiated from THRB biochemically with a more significant FT3 elevation than FT4, resulting in a low FT4/FT3 ratio, which was not observed in this patient.[9] Bone profiles, including serum calcium, serum phosphate and alkaline phosphatase, as well as hemoglobin and creatinine kinase levels, can differentiate THR-alpha from THRB. THR-alpha is associated with both skeletal dysplasia, such as macrocephaly and epiphyseal dysgenesis, and normocytic normochromic anemia, which were not seen in this patient.[10] High circulating thyroid hormone results in tachycardia in the case of THRB and may be mistaken for Graves' disease. Although most patients with THRB are clinically euthyroid, some may manifest with goiter and intermittent tachycardia without other peripheral signs of thyrotoxicosis.[11] Moreover, the thyroid hormone antibodies are absent in THRB.

The differential diagnoses for high FT4 and TSH are assay interference, familial dysalbuminemic hyperthyroxinemia (FDH) and TSH-secreting pituitary tumor (TSHoma). Thyroid hormone assay interference can be ruled out by repeating TFT in a different laboratory with a different analysis method. It might be due to the presence of binding antibodies, which results in a falsely high FT4 or TSH. Serial TFT was sent to other laboratories, and the results were similar. FDH is a rare autosomal dominant disease whereby the thyroxine-binding capacity of mutated albumin increases, resulting in an abnormally high thyroid hormone without physical abnormalities.[12] MRI of the pituitary gland was normal, ruling out a TSH-secreting tumor of the pituitary gland.

THR-beta syndrome is inherited in an autosomal dominant, autosomal recessive or sporadic pattern with de novo mutations in 20% of the cases.[13] THR syndrome is caused by a defect in the THRB gene located at chromosome 3. In our patient, genetic testing for both the patient and father showed R243W gene mutation in exon 7, whereby normal arginine-243 has been substituted by tryptophan. This mutation was reported in 1996 (2), with most THRB mutations identified between exons 7 and 10.[14]

Clinical manifestations of THR can be highly variable. Canadas et al.,[15] reported a 4-year-old girl who presented with hyperthyroidism and goiter, causing compressive symptoms. Her TFT showed an elevation of both thyroid hormone and TSH. Genetic testing revealed a mutation of the thyroid beta receptor gene, E445X. A total thyroidectomy was performed. However, her TSH did not decrease despite taking high-dose levothyroxine (175 mcg daily). Triiodothyronine (200 mcg every other day) was added to improve her response. Another case was reported by Tong et al., whereby a 15-year-old girl with attention-deficit hyperactivity disorder (ADHD) presented with oligomenorrhea and a positive family history of hyperthyroidism in her mother and maternal uncle.[16] Her TFT showed high TSH, normal FT4 with positive TPO. She was diagnosed with Hashimoto's thyroiditis and was given levothyroxine. However, the TFT showed persistent derangement. Family screening showed that her mother had similar TFT results. Genetic testing revealed a heterozygous mutation in c8303 C>G in the THRB gene, thus confirming the diagnosis of THRB.

The majority of patients with THR syndrome do not require treatment. Normalization of thyroid hormone levels is not the sole treatment goal. Treatment with conventional antithyroid drugs such as carbimazole may result in drug toxicity, particularly if the attending physician prescribed the dose used for patients with thyrotoxicosis.[17] Beta-blockers can be used for symptomatic treatment of adrenergic symptoms such as palpitation/tachycardia and breathlessness.[11] Thyroid hormone replacement may be initiated for patients with THR-alpha who manifest symptoms of hypothyroidism, especially during infancy.[3] The recommended specific treatment for thyrotoxicosis is triiodothyronine acetate (TRIAC), which inhibits TSH activity, thereby reducing the hypermetabolic state without affecting peripheral tissue activity.[17] However, TRIAC was not available in our country. Surgical removal of the thyroid gland and ablation treatment should not be done as it complicates subsequent management and monitoring.[17] In this patient, we provided symptomatic treatment such as a beta-blocker for palpitations and regular monitoring of her TFT. During subsequent follow-ups, her development milestones had been catching up but were still delayed compared to her peers. She can walk independently, speak in phrases, feed herself and put on clothes. At around 7 to 8 years old, her weight is in the 5th centile while her height is in the 10th centile (Figure 3). Her TFT still showed elevated FT4 and TSH (Table 1).

CONCLUSION

Clinical manifestations of THR syndrome are nonspecific and diverse. Early diagnosis is challenging. However, with

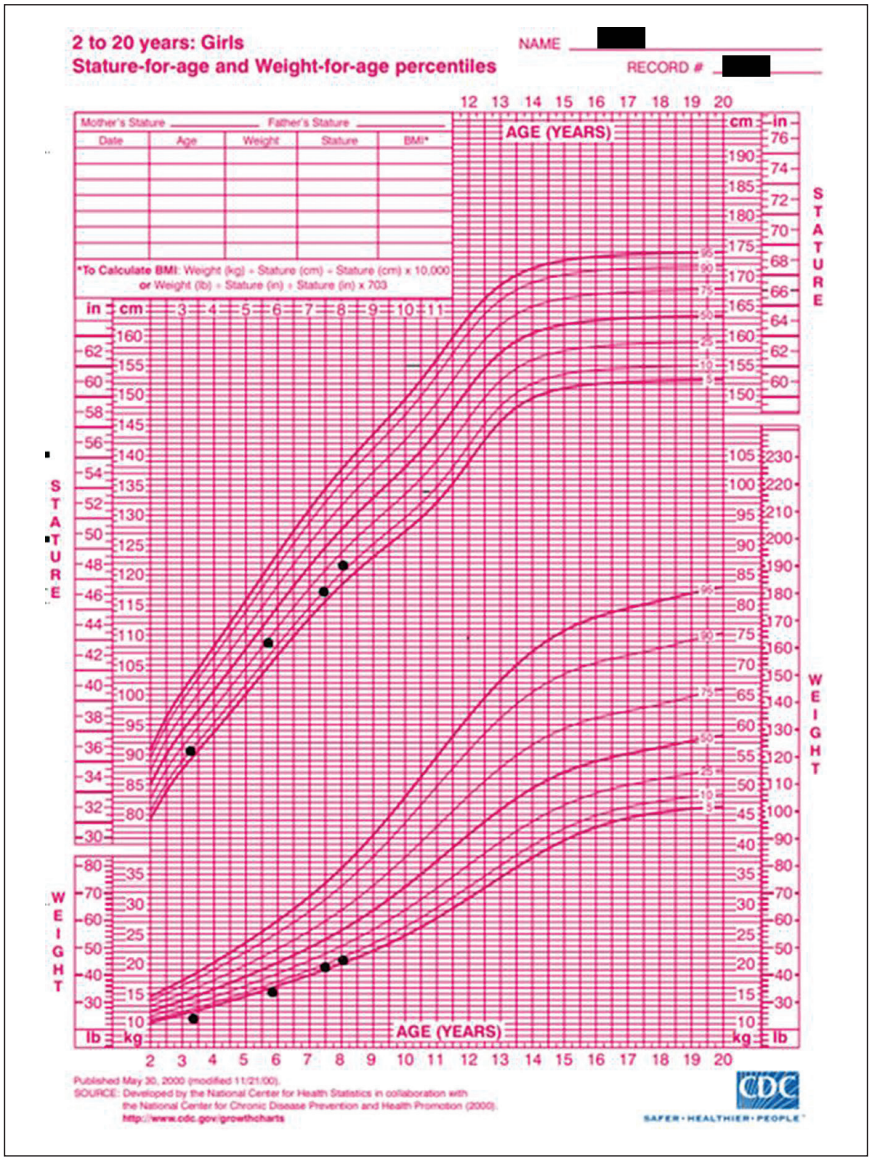

Figure 3. Growth chart. Centers for Disease Control Prevention. National Center for Health Statistics. Downloadable charts.

https://www.cdc.gov/growthcharts/data/set1clinical/cj41c022.pdf.

proper biochemical or hormonal tests and genetic testing, diagnosis can be made earlier to institute appropriate treatment.

Ethical Consideration

Patient consent was obtained before submission of the manuscript.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement

JCO: Writing – original draft preparation, Writing – review and editing; **WMHWO:** Resources; **TSTI:** Investigation; **KC:** Investigation; **SH:** Writing – review and editing, Supervision.

Author Disclosure

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Datasets generated and analyzed are included in the published article.

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CASE REPORT

Windswept Deformity: A Rare Skeletal Manifestation in an Adolescent with Primary Hyperparathyroidism

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Abstract

Primary hyperparathyroidism (PHPT) in adolescents is rare and has severe manifestations as compared to adults. Skeletal involvement in primary hyperparathyroidism in the form of deformities like genu valgus, genu varus and cubitus varus is rare and limited to case reports and case series. There is only one case of genu varus with genu valgus on the contralateral extremity (windswept deformity) that has been reported to date in the literature. We report the case of a 19-year-old male who presented with isolated progressive bending of his legs at the knee (windswept deformity) for three years. He was found to have hypercalcemia, hypophosphatemia, high alkaline phosphatase, high intact parathyroid hormone (iPTH), normal 25-hydroxy vitamin D level and a normal kidney function test. A diagnosis of primary hyperparathyroidism was made. On imaging studies, a left inferior parathyroid adenoma was localized and was successfully removed surgically. Serum calcium and iPTH normalized post-operatively. The patient is being planned for corrective osteotomy after stabilization of alkaline phosphatase levels.

Key words: primary hyperparathyroidism, skeletal manifestations, genu valgus, genu varus, wind-swept deformity

INTRODUCTION

Primary hyperparathyroidism (PHPT) is rare in pediatric and adolescent-aged individuals with an incidence of 1/300,000 live births/ year and a prevalence of 3-5/100,000.[1] Single gland disease is the most common presentation that is similar to adults. It can also be a part of syndromes like multiple endocrine neoplasia (MEN).[2] In developed countries, it is diagnosed earlier because of more frequent serum calcium screening.[3] However, florid manifestations, including skeletal manifestations with deformities, are still seen in developing countries like India.[4] Skeletal deformity, especially windswept deformity (genu valgus in one limb and genu varus in contralateral limb), in PHPT is rare. The common differentials for this deformity are rickets (hypophosphatemia, vitamin D deficiency and calcium deficiency), skeletal dysplasia, chronic fluoride toxicity, distal renal tubular acidosis, renal osteodystrophy and, rarely, trauma.[5] If PHPT and associated deformity are not diagnosed early and promptly treated, this can lead to further deformities, gait abnormalities, limb shortening and osteoarthritis.[6] We report a 19-year-old male who presented with windswept deformity due to primary hyperparathyroidism from a parathyroid adenoma. Early diagnosis of PHPT is important to prevent significant morbidity in the form of end-organ damage and mortality.

CASE

A 19-year-old male presented with progressive bending of his legs at the knee over three years' duration which his parents attributed to excess body weight. The deformity worsened in the last six months for which he was brought to the orthopedic outpatient department (OPD) of our institute. He had no history of pain, swelling, or trauma to the knees. On laboratory investigations, he was found to have hypercalcemia which prompted an endocrinology service referral. He had achieved developmental milestones at the appropriate age. His two younger brothers and one elder sister did not report any similar deformities. There was no significant personal or family history of renal calculi or multiple endocrine neoplasia (MEN)-related disorders. On examination, his height was 167 cm (10^{th}-25^{th} percentile), weight was 77 kg (75^{th}-97^{th} centile) with a computed body mass index of 27.6 kg/m^2 (obese). He was Tanner's stage 5 in pubertal development. A windswept deformity was noted in the lower limbs (genu valgus in the right lower limb, and genu varus in the contralateral lower limb) (Figure 1). Clinically, there were no other skeletal deformities that were noted. Laboratory evaluation revealed elevated albumin-corrected serum calcium, low serum phosphate, high alkaline phosphatase, high intact parathyroid hormone (iPTH), normal 25-hydroxyvitamin D levels and a normal

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Figure 1. Windswept deformity (genu valgus on the right lower limb and genu varus on the left lower limb) on clinical examination of the patient.

Table 1. Baseline laboratory profile at the time of endocrine evaluation

Parameters	Patient's pre-operative values	Reference range
Serum creatinine (mg/dL)	0.5	0.66 -1.25
Serum albumin (g/dL)	4.2	3.5-5
Serum albumin-adjusted total calcium (mg/dL)	11.9	8.4-10.2
Serum phosphate (mg/dL)	2.7	2.7-4.7
Serum alkaline phosphatase (U/L)	2929	38-126
Serum intact Parathyroid Hormone (iPTH) (pg/ml)	720	7.5-53.5
Serum 25-hydroxyvitamin D (ng/ml)	28.8	30-100
Serum magnesium (mg/dL)	2.4	1.6-2.5

kidney function test (Table 1). The results were consistent with a diagnosis of primary hyperparathyroidism. Ultrasonography of the neck showed a hypoechoic lesion in the inferior pole of the left thyroid lobe suggestive of a parathyroid adenoma. Technetium (^{99m}TC) tetrofosmin scan revealed a left inferior parathyroid adenoma (Figure 2). There was no evidence of gallbladder or renal calculi on ultrasonography. Genetic testing for MEN syndromes could not be done because of non-availability at our Institute. A skeletal survey revealed valgus deformity at the right knee joint and varus deformity at the left knee joint (Figure 3). There was generalized osteopenia with subperiosteal resorption of proximal phalynx of the first finger, middle phalanx of the second, third, fourth and fifth fingers of both hands and brown tumor in the middle phalynx of the fourth finger of the left hand and and acro-osteolysis in the distal phalanx of the second, third, and fourth finger of the left hand (Figure 4). Interestingly, radiographs of both hands revealed open physes of both the radius and ulna. Subsequently, the patient underwent a left inferior parathyroidectomy. Repeat serum intact PTH level 10 minutes after left inferior parathyroid gland excision decreased by >50% from 720 pg/mL to 46.2 pg/mL, suggestive of cure. Histopathology confirmed the excised tissue to be a parathyroid adenoma. Postoperatively, he developed tingling sensations in the perioral area with positive Chvostek's sign. Laboratory evaluation showed total calcium of 7.6 mg/dL (albumin-corrected calcium of 7.5 mg/dL) and serum phosphate of 2 mg/dL possibly because of post-parathyroidectomy hungry bone syndrome. Treatment with intravenous calcium gluconate and oral calcitriol was given for two days. From the third postoperative day onwards, treatment with oral calcium carbonate 2 grams per day and oral calcitriol 0.5 micrograms twice a day was continued. The serum intact PTH on day seven of surgery was 53 pg/mL and albumin-corrected total calcium was 9.6 mg/dL. Monthly cholecalciferol (60000 IU) and daily calcium carbonate supplementation were continued. Corrective osteotomy is being planned by the orthopedic team after stabilization of the serum alkaline phosphatase level. In the meantime, he has been advised lifestyle modification for weight reduction to decrease mechanical pressure on lower limbs to prevent worsening of deformity.

DISCUSSION

Primary hyperparathyroidism in children and adolescents is uncommon. In addition, because of nonspecific signs and symptoms of the disease, healthcare professionals may fail to check serum calcium levels which will further delay the diagnosis and predispose patients to present with end-organ pathology.[7] Renal stone disease, bone involvement, and nephrocalcinosis are among the most common end-organ pathologies in pediatric patients.[8] Skeletal diseases associated with PHPT include bone pain, osteopenia, fractures or lytic bone lesions and bone deformities.[9]

Skeletal deformities in the form of genu valgus, genu varus, genu valgus on one side and genu varus on the contralateral side (windswept deformity) and cubitus valgus from primary hyperparathyroidism in children and adolescents are uncommon. Though they have been reported in the literature, the presence of these deformities should be assessed with a high index of suspicion for metabolic causes and should necessitate further workup. The typical causes of windswept deformity are rickets (hypophosphatemic, vitamin D deficiency, calcium deficiency), skeletal dysplasias, chronic fluoride toxicity, distal renal tubular acidosis, renal osteodystrophy and, rarely, trauma.[5] Timely identification of its etiology and corresponding treatment is important. Treatment options, apart from treatment of the specific etiology, include surgical (corrective osteotomies, stapling) or conservative (plaster casting) approaches. If left untreated, it can lead

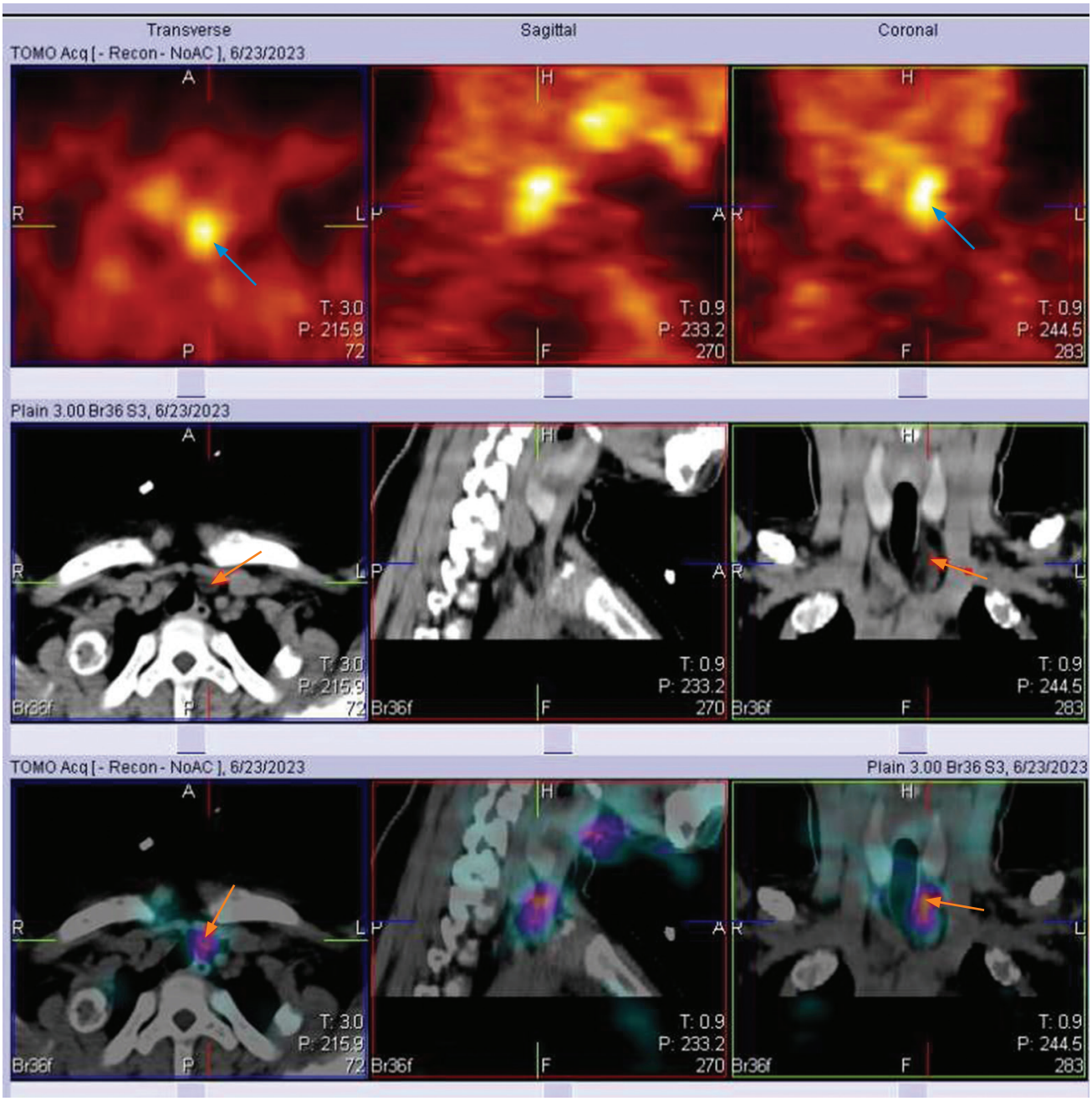

Figure 2. Technetium (^{99m}Tc) tetrofosmin and SPECT/CT scan showing tracer accumulation in the region of the left inferior parathyroid gland suggestive of left inferior parathyroid adenoma.

to further deformities, gait abnormalities, limb shortening and osteoarthritis.

To our knowledge, there are only 36 cases of genu valgus, one case of cubitus valgus, and one case each of genu varus and windswept deformity have been reported in the literature (Table 2). The age of presentation of cases presented ranged from 11 to 21 years. The pathogenesis of such deformities in this age group is unclear. However, it has been postulated that elevated parathyroid hormone can directly affect growth plate and bone remodeling during pubertal growth spurt.[10] We propose that the reported patient's excess body weight, in addition to the effect of PTH, may have contributed to the deformity.

In addition to genu valgus, other radiological features typical of primary hyperparathyroidism have also been reported in cases reported in the literature. These include osteopenia, subperiosteal resorption of phalanges, acro-osteolysis, brown tumor, and salt-and-pepper appearance of a skull.[9-33] 16 patients had features of rickets clinically or radiologically. Our patient presented with windswept deformity in the lower limb without other symptoms due to hypercalcemia and no characteristic features of rickets. He had radiological features of primary hyperparathyroidism similar to the cases reported in the literature. He had a single parathyroid adenoma similar to all reported cases in the literature (Table 2).

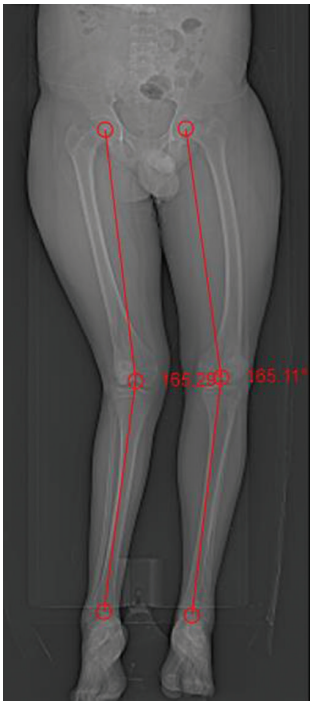

Figure 3. Radiograph of bilateral tibia and femora showing windswept deformity at the knee, characterized as genu varus on the left side (15°) and genu valgus (15°) on the right side.

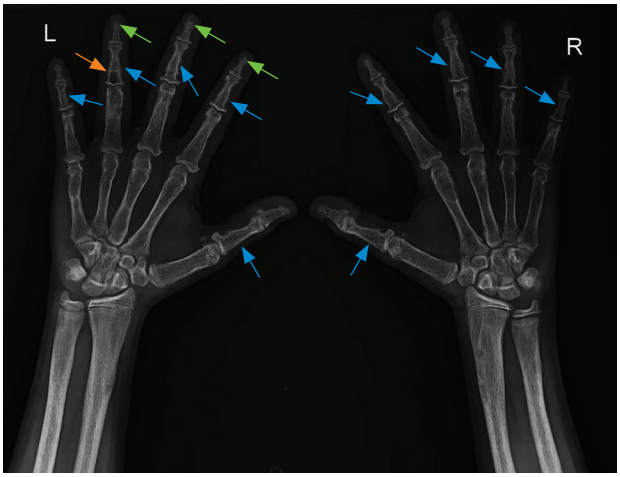

Figure 4. Radiograph of both hands showing generalized osteopenia with subperiosteal resorption *(blue arrows)* of the middle phalanx of the first, second, third, fourth and fifth fingers of both hands, brown tumor *(orange arrow)* in the middle phalanx of the fourth finger of the left hand and acro-osteolysis *(green arrows)* in the distal phalanx of the second, third and fourth finger of the left hand.

Table 2. Summary of reported cases with primary hyperparathyroidism and skeletal deformities

Author, Year	Age (years), Sex	Clinical feature	Etiology
De Silva et al., 2023[11]	18, M	Bilateral (B/L) genu valgus, short stature	LIPA
Dikova et al., 2021[12]	12, F	B/L genu valgus, inability to walk	RIPA
	15, F	Left genu valgus, waddling gait	LIPA
Boro H et al., 2022[9]	15, F	Bone pain, genu valgus	LIPA
	17, F	Fatigue, genu valgus, bone pain	LIPA
	19, F	Bone pain, right humerus fracture, reflux symptoms, genu valgus	RIPA
	15, M	Fatigue, genu valgus, renal stones	LIPA
	17, M	Bone pain, genu valgus, cubitus valgus, constipation	RIPA
Lee et al., 2021[13]	15, M	B/L genu valgus, short stature	LIPA
Yanrismet Y et al., 2019[14]	13, M	B/L genu valgus, bone pain, muscle weakness	RIPA
Rao KS et al., 2019[15]	12, F	B/L genu valgus, renal stones	RIPA
Paruk IM et al., 2019[16]	17, M	B/L genu valgus, short stature	LIPA
	13, M	Left genu valgus, right genu varus	RIPA
Khan et al., 2019[17]	17, M	B/L genu valgus, short stature, muscle wasting	LIPA
George GS et al., 2019[18]	15, M	B/L genu valgus, Slipped capital femoral epiphysis	RIPA
Kamath SP et al., 2018[19]	11, F	Fatigue headache, B/L genu valgum	LIPA
	12, M	B/L genu valgum, renal stones	LIPA
Pradhan R et al., 2018[20]	15, F	B/L genu valgum, widening of wrists	LIPA
	15, F	B/L genu valgum fractured left clavicle, kyphosis, rachitic rosary	-
	11, M	B/L genu valgum, proximal muscle weakness	-
Arambewela MH et al., 2017[21]	12, F	B/L genu valgus	RIPA
Zil-E-Ali A et al., 2016[22]	14, F	Short stature, B/L genu valgus, pectus carinatum, scoliosis	RIPA
Sharma S et al., 2016[23]	15, F	Genu valgus, pectus carinatum	LIPA
Ganie M et al., 2016[24]	14, M	B/L genu valgus, bone pain	LIPA
	14, M	Genu valgus, Widening of wrists	Right lower neck (ectopic) adenoma
	15, M	Genu valgus, bone pain, fracture of right tibia	RIPA
Ramkumar S et al., 2014[10]	16, M	Leg pain, B/L genu valgus, generalised arthralgia, polyuria	LIPA
	13, M	B/L genu varus, Nausea, abdominal pain	RIPA
Ratnasigam J et al., 2013[25]	15, F	B/L genu valgus	Right parathyroid adenoma
Dutta D et al., 2013[26]	12, F	B/L genu valgus, short stature, flat feet	RIPA
Walczyk A et al., 2011[27]	15, M	B/L genu valgus, seizures	RIPA
Harman CR et al., 1999[8]	14, F	B/L genu valgus	-
Menon PS et al., 1994[28]	14, F	B/L genu valgus, short stature, renal stones, rachitic rosary	LSPA
Kauffmann C et al., 1993[29]	13, F	B/L genu valgus	LIPA
Rapaport D et al., 1986[30]	15, F	B/L genu valgus, renal stones	RIPA
	15, M	B/L genu valgus, renal stones	RIPA
Lloyd HM et al., 1965[31]	14, M	B/L genu valgus, weakness, scoliosis	LIPA
Balch HE et al., 1953[32]	21, F	B/L genu valgus, vomiting, clubbing, rib tenderness	LIPA
Mcclure RD et al., 1945[33]	14, F	B/L genu valgus	LIPA

LIPA – Left inferior parathyroid adenoma, RIPA – Right inferior parathyroid adenoma, LSPA – Left superior parathyroid adenoma, M – Male, F – Female, B/L – Bilateral

Surgical removal of the involved parathyroid gland is the mainstay of treatment of PHPT.[7] Our patient underwent a successful parathyroidectomy, similar to the reported cases, and he is currently being planned for osteotomy for windswept deformity correction.

CONCLUSION

We have reported a rare case of skeletal manifestation in the form of genu varus in one limb with genu valgus in the contralateral limb (windswept deformity) in a male adolescent secondary to PHPT. As PHPT is rare in adolescents and may mimic other conditions, like rickets, when the disease manifests in the form of skeletal deformities, misdiagnosis and inappropriate management may inevitably lead to further aggravation of skeletal system involvement. When children and adolescent patients present with skeletal manifestations and deformities, this should prompt the immediate diagnostic evaluation by measuring serum calcium, phosphorus and PTH levels to make a timely diagnosis and treatment. Prompt treatment may eventually lead to a cure and prevent end-organ damage. Moreover, additional research is needed to further understand the pathogenesis of skeletal deformities in PHPT in children and adolescent patients.

Caregiver's Perspective

Before coming to JNMC and DMIHER at Sawangi, my wife and I were overwhelmed. Our son's diagnosis left us confused, afraid and emotionally drained. The financial burden added a heavy weight to our worries. Thankfully, the doctors here at JNMC and DMIHER provided us with the cooperation, proper evaluation, and effective management we desperately needed. We are happy to say that our son's treatment is progressing well.

Ethical Consideration

Patient consent was obtained before submission of the manuscript.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement

PF: Conceptualization, Writing – original draft preparation; **BJ:** Writing – review and editing; **SS:** Writing – review and editing, Supervision.

Author Disclosure

The authors declared no conflict of interest.

Data Availability Statement

No datasets were generated or analyzed for this study.

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None.

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CASE REPORT

An Unusual Case of Adrenocortical Carcinoma with Multiple Facets

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Abstract

Adrenocortical carcinoma (ACC) is a rare malignant tumour from the adrenal cortex. Half of the cases are functional, with ACTH-independent autonomous cortisol production being the most common. It is rare for ACC to present with markedly elevated metanephrine levels, characteristic of pheochromocytoma. We report a case of a large functioning adrenal tumour with overlapping biochemical features of ACC and pheochromocytoma. Biopsy confirmed the histopathological diagnosis of metastatic ACC.

Key words: adrenocortical carcinoma, pheochromocytoma, urine fractionated metanephrines

INTRODUCTION

Adrenocortical carcinoma (ACC) and pheochromocytoma are both rare tumours with incidence ranging from 0.7 to 2.0 per million per year for ACC and 2 to 8 per million per year in combined cases of pheochromocytoma and paraganglioma (PPGL).[1,2] It is often difficult to differentiate ACC from pheochromocytoma based on imaging alone. ACC and pheochromocytoma share common radiological characteristics such as large size, high attenuation values on unenhanced CT and inhomogeneity with areas of haemorrhage or necrosis with or without calcifications, which makes diagnosis challenging. A comprehensive endocrine work-up is helpful to differentiate one entity from another. This is important as the management and prognosis of the two diseases differ. One distinguishing feature is that pheochromocytomas secrete catecholamines, whereas most ACCs, if functioning, can secrete various adrenocortical hormones, including cortisol, sex steroids or rarely mineralocorticoids. Cortisol excess in functioning ACC is usually non-ACTH dependent, whereas pheochromocytoma can be associated with ACTH-dependent Cushing or ectopic ACTH syndrome (EAS). Pheochromocytoma must be ruled out in the initial evaluation of an adrenal mass to prevent a potentially life-threatening pheochromocytoma crisis before undertaking any invasive procedure. Furthermore, autonomous cortisol secretion from a functioning ACC or ectopic ACTH secretion from pheochromocytoma will require steroid replacement perioperatively during resection to prevent adrenal crisis.

We herein report a case of a large adrenal tumour with an elevated normetanephrine level but with concurrent androgen excess and ACTH-dependent subclinical Cushing syndrome, in which the diagnosis was finally clinched through tissue biopsy.

CASE

A 49-year-old Malay female with no known medical illness presented with a two-month history of abdominal pain and distension associated with nausea, vomiting and unintentional weight loss. She had been married for 25 years but remained nulliparous. At the time of presentation, she was amenorrhoeic for almost a year. She denied paroxysmal symptoms and had no significant family history of neuroendocrine tumours or solid organ tumours. She did not appear Cushingoid or hirsute. On examination, she had a normal BMI of 22 kg/m^2 (height 1.46 m, weight 47.9 kg), blood pressure was 141/76 mm Hg, and pulse rate was 97 beats per minute. Physical examination was significant for hepatomegaly.

Her initial bedside ultrasound showed multiple liver masses. A multiphase CT scan of the liver was subsequently done (Figure 1A), which revealed multiple heterogeneous masses in the right liver lobe with necrotic components, the largest in segment VII/VIII, measuring 9.3 x 10.3 x 10.4 cm. Another liver mass in segment V/VI measuring 8.9 x 9.2 x 8.5 cm, associated with the capsular breach and surrounding perihepatic hemoperitoneum suggestive of tumoral rupture. A large heterogenous, lobulated left

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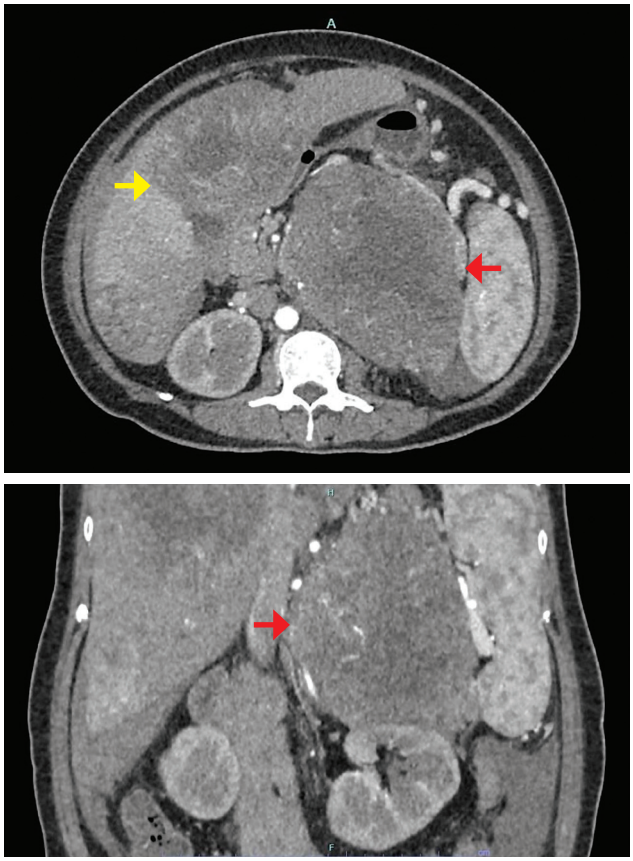

Figure 1. (A) Large heterogenous left adrenal mass *(red arrow)* with central hypodensity and multiple heterogenous masses *(yellow arrow)* in the right liver lobe (CT Abdomen axial view in arterial phase). **(B)** Left adrenal mass *(red arrow)* displaced the left kidney with no clear fat plane and abuts the spleen (CT abdomen coronal view in arterial phase).

suprarenal mass was visualized, measuring 9.9 x 12.7 x 13.5 cm with hypodense areas, which may represent a necrotic component (CT attenuation values: 39 HU on plain, 61 HU on arterial phase, 60 HU on portovenous phase and 59HU on delayed phase). The suprarenal mass showed some patchy arterial enhancement, displaced the left kidney with no clear fat plane and abutted the spleen (Figure 1B) . The right adrenal gland and pancreas appeared normal. Based on the imaging characteristics of the adrenal lesion, adrenocortical carcinoma was highly possible, but pheochromocytoma could not be ruled out.

Her subsequent hormonal workup revealed a basal 8 a.m. serum cortisol of 491 nmol/L, which was not suppressible with an overnight 1 mg dexamethasone suppression test (cortisol 474 nmol/L) or 48-hours, 2 mg/day low-dose dexamethasone suppression test (cortisol 508 nmol/L). Her DHEA-S level was markedly elevated at >27.1 micromol/L (reference range: 0.96-6.95), and her testosterone was 5.120 nmol/L (reference range: 0.29-1.67) with suppressed gonadotropins. The excess of both the cortisol and sex steroid hormones was consistent with the suspicion of a functioning ACC. Unexpectedly, ACTH levels were non-suppressed at 6.2 pmol/L and repeatedly within

Table 1. 24-hour urine metanephrines

Laboratory parameters	Value	Unit	Reference
Urine volume	0.97	L	
Urine PH	2.00		
Urine creatinine	8.83	mmol/24 h	
Normetanephrine	6.30	µmol/day	0.00 - 2.13
Metanephrine	0.40	µmol/day	0.00 - 1.62
3-methoxytyramine	1.80	µmol/day	0.10 - 1.79

the normal range, 5.4 pmol/L (normal 1.6-13.9) using the Roche Elecsys electrochemiluminescence immunoassay (ECLIA) method, suggesting ACTH-dependent Cushing syndrome. Adding to the diagnostic dilemma, her 24-hour urine metanephrine by liquid chromatography with an electrochemical detection method showed elevated normetanephrine levels three times above the upper reference limit (Table 1). The sample collection was strictly done under careful instructions, ensuring the absence of interfering drugs and significant physiological stress. Other than an elevated LDH 1287 U/L (reference range: 135-214) and ALP 601 U/L (reference range: 35-104), other blood parameters such as complete blood count, blood glucose, renal profile, electrolytes, including corrected calcium and liver function, were within normal limits.

Given the raised urine normetanephrine level and inability to rule out pheochromocytoma, she was started on an alpha-blocker, terazosin 1 mg ON. With up-titration of terazosin to 3 mg ON, she achieved normotension (systolic BP ranged 118-130 mm Hg, diastolic BP 53-69 mm Hg). A beta blocker was added later, maintaining her heart rate between 84 to 91 beats per minute without tachycardia. CT of the neck and thorax was done to look for extra-adrenal tumours or paragangliomas, but no suspicious lesions were detected. Similarly, MIBG scintigraphy did not show any MIBG-avid disease. A multidisciplinary team discussion concluded with the decision to do a biopsy of the adrenal and liver lesions for a likely inoperable disease and the need for histopathological diagnosis for oncologic planning. The diagnosis of metastatic adrenocortical carcinoma was still highly probable at the time of discussion due to the biochemically confirmed nature of mixed hormonal hypersecretion of both cortisol and androgen of the tumour. The biopsy was done after the adrenergic blockade.

Her liver and adrenal mass biopsy results later confirmed the histopathological diagnosis of metastatic adrenocortical carcinoma (Figures 2A and B), with immunohistochemical staining showing diffuse strong positivity for synaptophysin and inhibin (Figure 3) and focal positivity for Melan A. The cells stained negative for Chromogranin A, ruling out pheochromocytoma. ACTH staining was not available locally. Limited by the small tissue sample, the pathologist could not provide further information to prognosticate based on the Weiss score, Ki67 index or nodal status. She was ENSAT stage IV, nevertheless, due to the evidence of liver metastasis. As her disease was advanced and complete resection was not possible, she was offered mitotane with chemotherapy (etoposide/carboplatin).

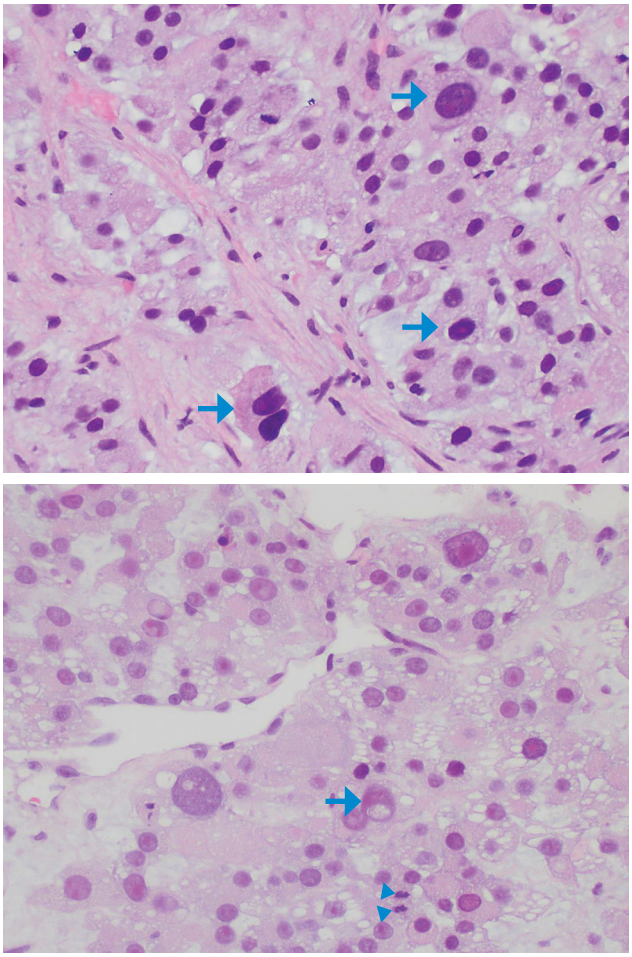

Figure 2. (A) Tumour exhibits large cells displaying large round pleomorphic hyperchromatic nuclei, prominent eosinophilic nucleoli and abundant granular cytoplasm (H&E, 40x). **(B)** Frequent intranuclear inclusions *(blue arrow)* and mitoses *(blue arrow heads)* (H&E, 40x).

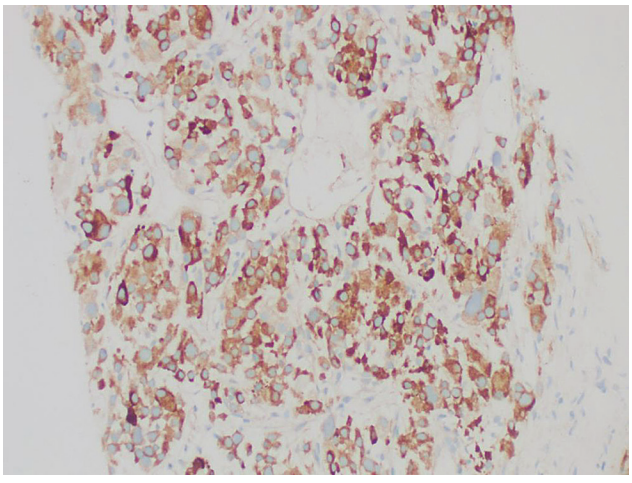

Figure 3. Synaptophysin and Inhibin stain with strong diffuse positivity (20x).

DISCUSSION

It is uncommon for ACC to present with markedly elevated metanephrine levels and even more unusual for the same disease entity to show ACTH-dependent Cushing syndrome (CS) with androgen excess.

Plasma free or urine fractionated metanephrines have high diagnostic sensitivity and specificity. Patients who tested positive should be followed up appropriately, considering both clinical presentation and degree of metanephrine elevation.[3] The isolated elevation of urine normetanephrine 3-fold above the upper limit was significant. However, the MIBG scan, pathological findings and immunohistochemical staining have ruled out the definitive diagnosis of pheochromocytoma. Therefore, the increased level of 24-hour urinary normetanephrine may be a false positive in this case. False positive biochemical test results for pheochromocytoma are common and present particular problems because of the low prevalence of the disease.[4] The diagnostic cut-offs for most 24-hour urinary fractionated metanephrine assays are based on normal ranges derived from a normotensive volunteer reference group, which can result in a high rate of false-positive results.[5] For this reason, tests should be repeated to confirm the results.[6]

A literature review revealed several possible similar cases of adrenal cortical tumours with increased metanephrines and catecholamine levels, labeling them as metanephrine-producing adrenocortical carcinoma[7] and so-called pseudo-pheochromocytoma.[8]

The unsuppressed ACTH level was an unexpected finding in this case. If the plasma ACTH concentration is above 20 pg/mL (4.4 pmol/L) in a patient with sustained hypercortisolism, one can assume that cortisol secretion is ACTH-dependent (i.e., due to pituitary disease or ectopic ACTH or corticotropin-releasing hormone [CRH] secretion). However, patients with ACTH-independent causes of Cushing syndrome who have cyclic or mild hypercortisolism (and consequently lack suppression of normal corticotrophs) may have normal ACTH values, falsely indicating an ACTH-dependent condition.[9]

Of note is that ACTH immunoassays are vulnerable to assay interference, which gives rise to discordant biochemical results from the clinical pictures. ACTH assays are also burdened by high variability and often fail to identify patients with suppressed ACTH secretion correctly.[10] Based on the results of an Italian multicentre study, it was found that plasma ACTH concentrations were detectable in 58% of patients with ACTH-independent CS, and 28% fell within the normal range among this group of patients using the radioactive immunoassay (RIA) or immunoradiometric assay (IRMA) method.[11] Another multi-center study conducted by Giraldi et al. found that 40% of plasma ACTH measurements fell into the normal range using chemiluminescent immunometric assay (CLS) and IRMA in patients whose ACTH secretion should

be suppressed. This raises the question of whether this phenomenon could be due to technical problems or if the pathophysiology of glucocorticoid negative feedback and secondary adrenal insufficiency should be revisited.[10] In this case, plasma ACTH was measured using the newer electrochemiluminescence ACTH immunoassay (Roche Diagnostics). Although rarely reported with the newer ECLIA method, it is worthwhile to exclude assay interference when encountering biochemical results discordant with the clinical presentation. Communication with the pathologists on further steps to eliminate analytical errors would be useful in identifying the mechanism of CS in this case.

Dilrukshi et al., described a case of ACC-associated aberrant ACTH production in which the tumour cells expressed granular cytoplasmic positivity for ACTH, having a plasma ACTH level of 40.3 pg/ml (8.87 pmol/L).[12] Law A et al., also reported 2 cases of functioning ACCs with unsuppressed ACTH concentrations despite having glucocorticoid excess. Both cases showed no stainable ACTH with anti-ACTH antibody on histology, and the exact pathophysiology was unknown.[13]

Rarely, ACC can be associated with multiple endocrine neoplasia type 1 (MEN1). Hypercortisolism in the context of MEN1 can result from pituitary, adrenal or thymic neuroendocrine tumours and can therefore reflect either ACTH-dependent or ACTH-independent pathophysiologies.[14] The incidence of ACC is approximately 1% in MEN1 patients and 13% in MEN1 patients with adrenal tumours larger than 1 cm.[15] The diagnosis of MEN1 is less likely in this case given that the patient was normocalcemic, which makes primary hyperparathyroidism unlikely, and the pancreas was normal on imaging. However, an MRI of the pituitary may help rule out a pituitary adenoma, especially in cases of ACTH-dependent CS. A high-dose dexamethasone suppression test and CRH stimulation test may be helpful in the work-up of unsuppressed ACTH levels, as was seen in this case . Cases of subclinical CS with ectopic ACTH have been described in mixed corticomedullary tumour (MCMT) of the adrenal gland by Kimura et al., MCMT is an extremely rare tumour characterized by an admixture of steroidogenic cells and chromaffin cells in a single tumour mass, producing adrenocortical hormones and catecholamines.[16] As surgical resection was not done in this case, there remain unanswered questions if the biopsy samples are fully representative of the underlying disease aetiology.

An adrenal biopsy is generally not recommended for suspected ACCs due to the risk of tumour dissemination and significant risks, such as haemorrhage. Exceptions are conditions where the disease is inoperable, and confirmation of diagnosis is needed for oncologic management, as part of a clinical trial or suspected metastasis from an extra-adrenal malignancy.[17] The presence of metastatic disease in this case precluded surgery, and histological proof was pertinent to distinguish ACC from pheochromocytoma with certainty as management would differ depending on the disease aetiology. MIBG scintigraphy, despite being the gold-standard diagnostic tool for pheochromocytoma, can be negative in poorly differentiated or metastatic pheochromocytomas.[18] A retrospective review on transcutaneous adrenal biopsy done in Stage I to III ACCs showed that biopsy did not significantly affect patient outcomes in terms of recurrence-free or overall survival but may only be helpful in the right setting.[19]

CONCLUSION

The myriad biochemical features of ACC, some of which overlap with pheochromocytoma, can make it challenging for clinicians to arrive at an accurate diagnosis. Close collaboration of a multidisciplinary team comprising of endocrinologists, pathologists, radiologists, endocrine surgeons and oncologists is needed to facilitate accurate diagnosis and appropriate management in such unique cases with multiple adrenocortical hormone excess with markedly elevated metanephrines.

Ethical Consideration
Patient consent was obtained before submission of the manuscript.

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

CRediT Author Statement
JET: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **FHST:** Validation, Writing – review and editing, Supervision; **YCK:** Validation, Writing – review and editing, Supervision; **PLC:** Writing – review and editing, Supervision; **YY:** Investigation, Resources.

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CASE REPORT

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Cushing Disease in a Patient with Double Pituitary Adenomas Complicated with Diabetes Insipidus: A Case Report

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Abstract

Managing a patient with both pituitary hypersecretory and hyposecretory manifestations may be perplexing. We report a 14-year-old female who presented with weight gain, polyuria and polydipsia. Biochemical results were consistent with Cushing disease with central diabetes insipidus. Pituitary magnetic resonance imaging showed a right adenoma with stalk thickening. The immunohistochemistry staining of both adenomas was positive for adrenocorticotropic hormone, thyroid stimulating hormone, growth hormone and luteinizing hormone. Postoperatively, the patient developed panhypopituitarism with persistent diabetes insipidus. The coexistence of double adenomas can pose diagnostic and management challenges and is a common cause of surgical failure. Intraoperative evaluation is important in the identification of double or multiple pituitary adenomas in a patient presenting with multiple secretory manifestations.

Key words: double pituitary adenoma, Cushing disease, diabetes insipidus, adrenocorticotropic hormone-secreting pituitary adenoma

INTRODUCTION

Double or multiple pituitary adenomas are rare and are defined as two or more concurrent adenomas in the pituitary gland that differ morphologically or immunocytochemically.[1] The diagnosis of double or pituitary adenomas is based on histopathologic examination (HPE), surgical specimen (0.4-1.3%) and autopsy series (0.9 – 2%) because identification by magnetic resonance imaging (MRI) or during surgery is difficult.[1-4] The tumours are usually microadenomas with an average size of 3 mm, or one is overshadowed by a co-existing but larger pituitary adenoma and is clinically silent.[5]

Presentations can be either non-functioning or functioning and rarely present with more than one hypersecretory syndrome due to pluri-hormonal adenomas (production of two or more hormones). We report a case of clearly separated adenomas presenting with Cushing syndrome (CS) with diabetes insipidus (DI). To the best of our knowledge, this is the youngest case in the recent literature of Cushing disease due to double pituitary adenomas (DPA), located within the anterior pituitary and infundibulum.

CASE

A 14-year-old female presented with a 10-month history of weight gain of 15 kg associated with hirsutism, polyuria, polydipsia and secondary amenorrhea of 9 months duration. Menarche occurred at twelve years of age. Signs of Cushing syndrome were present such as moon-like facies with mild plethora, truncal obesity, thin skin with easy bruising, purplish abdominal striae, proximal myopathy and hyperpigmentation over the creases. Her blood pressure was 158/89 mm Hg and her pulse rate was 96 beats per minute. Height was 153 cm, weight was 78.8 kg with body mass index of 33.6 kg/m^2 and waist circumference of 104 cm. Breast development and pubic hair distribution corresponded to Tanner stage 2. All other systems were unremarkable.

Her 24-hour urinary free cortisol was 512 nmol/24h (normal range: 57.7 – 806.6) and serum cortisol post low dose dexamethasone suppression test was 149 nmol/L. The diagnosis of adrenocorticotropic hormone (ACTH)-dependent CS was confirmed with ACTH level of 45.82 pg/ml (normal range: 7.2 – 63). The rest of the hormonal work-up is shown in Table 1. We suspected that the patient may have DI as shown by serum sodium of 151 mmol/L, serum osmolarity of 314 mOsm/kg, urine osmolarity of

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Table 1. Baseline (pre-operative) hormonal levels

Parameters	Results	Normal range
24 hours urinary free cortisol	512.2 (3.6 L of urine)	57.7-806.8 nmol/d
Overnight 1 mg dexamethasone suppression test	149	<50 nmol/L
ACTH	45.82	7.2-63 pg/ml
DHEAS	7.97	0.01-7.6 umol/L
Plasma Renin Activity	7.364	0.3-1.9 mg/ml/hr
Serum aldosterone	441.5	41.71-208.9 pg/ml (supine)
FSH	<0.3 IU/L	
LH	<0.1 IU/L	
Estradiol	133 pmol/L	
Testosterone	1.17	0.101-1.67 nmol/L
Cortisol	730	101-535.7 nmol/L
Prolactin	<0.3	1.4-24.2 ug/L
FT4	15.57	9-19.05 pmol/L
TSH	1.46	0.35-4.94 uIU/ml

ACTH: Adrenocorticotropic hormone; DHEAS: dehydroepiandrosterone sulfate; FSH: follicle-stimulating hormone; LH: luteinizing hormone; FT4: Free T4; TSH: thyroid stimulating hormone

83 mOsm/kg and urine sodium of 21 mmol/L. However, since there was no radiological evidence to suggest any stalk abnormalities, our team decided to proceed with both phase 1 and phase 2 of the water deprivation test. While preparing for the water deprivation test, the patient was advised to drink fluids whenever she felt thirsty, resulting in the normalization of the serum sodium on the day of the water deprivation test. The results of the water deprivation test were consistent with DI (Table 2). Pituitary MRI revealed a small focal area with delayed enhancement on dynamic sequence in the right pituitary gland measuring 3.0 x 2.0 mm (Figure. 1). On the mid-sagittal plane of the T1 post-contrast study, focal thickening of the pituitary stalk (3.9 mm) was suspicious of either an inflammatory cause or another mass lesion (Figure 2). The bilateral inferior petrosal sinus sampling with desmopressin stimulation demonstrated a right central to peripheral ACTH ratio of 7.42.

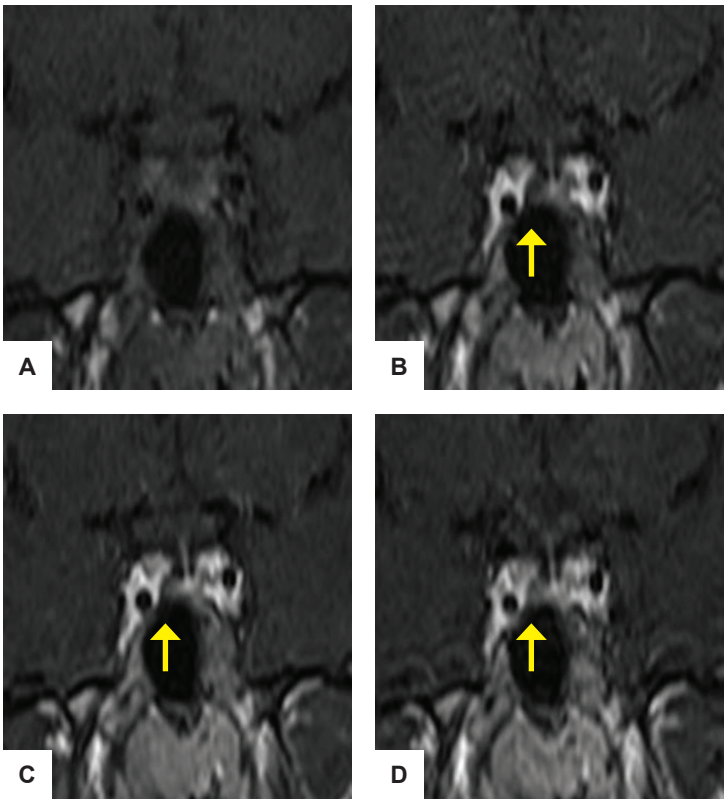

Figure 1. Pituitary MRI. High-resolution pre-contrast **(A)** and selected dynamic contrast enhance T1 **(B)**, **(C)**, and **(D)** at 39 seconds, 64 seconds and 89 seconds post-gadolinium respectively. The pituitary gland does not show any distinct lesion on the pre-contrast image. In the dynamic post-contrast sequence, a distinct area of delayed enhancement at the right side of the pituitary gland represents the microadenoma *(yellow arrow)*. A subtle displacement of the pituitary stalk to the left and mild inferior bulging of the sellar floor on the right side indicates the presence of a mass in the right pituitary gland.

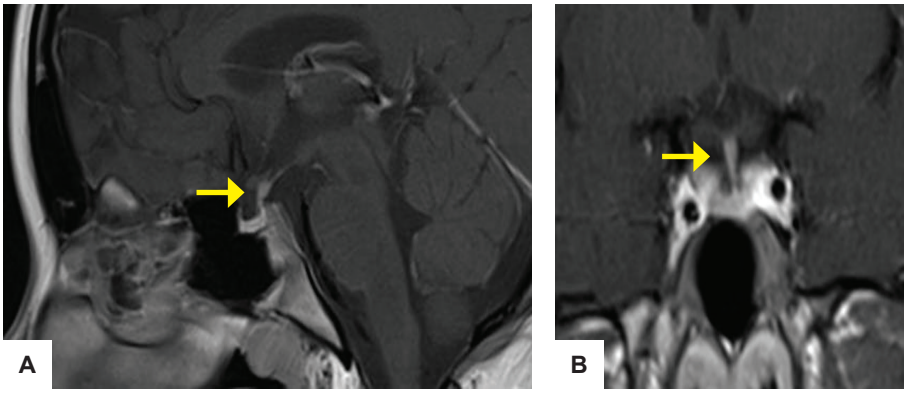

Figure 2. Pituitary MRI. T1 post-contrast in axial **(A)** and mid-sagittal **(B)** demonstrate homogeneous enhancement of the pituitary gland. On the mid-sagittal plane, at the optic chiasm level, a subtle enhancing pituitary stalk lesion, with focal thickening *(yellow arrow)* results in the loss of a normal tapering pituitary stalk.

Table 2. Water deprivation test results

Phase 1	10am	12pm	2pm
Weight (kg)	79.8	78.6	77.9
Serum Na (mmol/L)	138	138	144
Serum Osmol (mOsm/kg)	285	286	291
Urine Osmol (mOsm/kg)	60	60	74
Urine volume (ml)	200	250	300
*Subcutaneous desmopressin 2 ug administered at 4pm			
Phase 2	**4pm**	**6pm**	**8pm**
Weight (kg)	77.5	77.4	78.3
Serum Na (mmol/L)	141	138	137
Serum Osmol (mOsm/kg)	293	292	289
Urine Osmol (mOsm/kg)	76	100	221
Urine volume (ml)	310	200	150

She underwent transsphenoidal surgery (TSS) and intraoperatively, was noted to have two separate lesions containing cheesy material at the posterior aspect of the right pituitary lobe and the infundibulum. The right hypophysectomy resection extending to the midline with the infundibular mass removal was uneventful. Results of the HPE of both specimens were consistent with pituitary adenoma and both immunohistochemistry (IHC) stains were positive for ACTH, growth hormone (GH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH), and weakly positive for prolactin (PRL) and follicle-stimulating hormone (FSH) (Figures 3 and 4). On the second postoperative day, her ACTH, cortisol and Free T4 levels

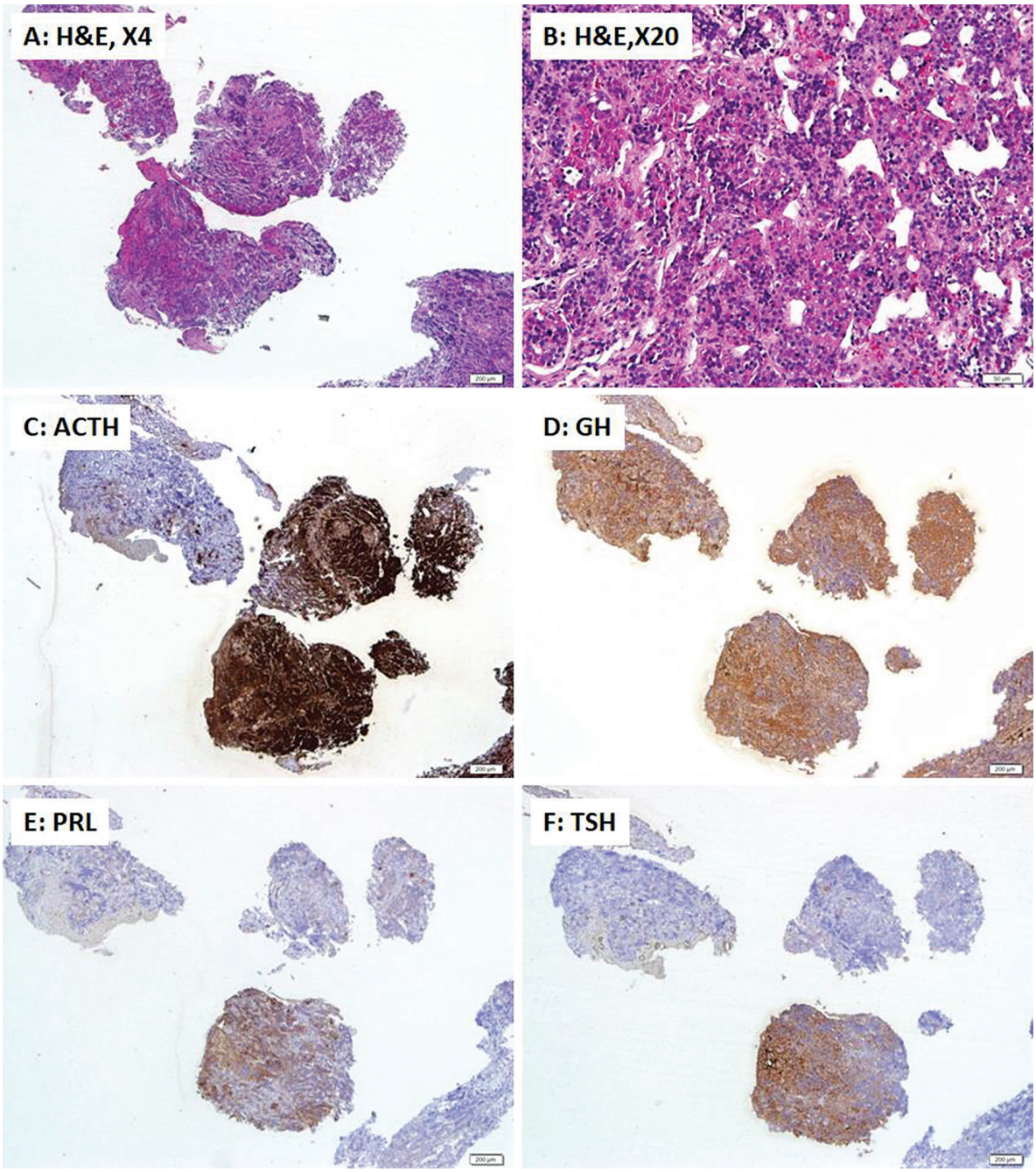

Figure 3. Pluri-hormonal pituitary adenoma located at the right lobe of pituitary. **(A)** (H&E, X4), **(B)** (H&E, X20) producing ACTH **(C)**, GH **(D)**, Prolactin **(E)** and TSH **(F)**.

were 4.2 pg/ml, <20 nmol/L and 7.36 pmol/L, respectively. Hence, anterior pituitary hormone replacement was initiated with oral hydrocortisone 10 mg and 5 mg at 8 am and 12 pm respectively, as well as oral levothyroxine 75 mcg daily. Her sublingual desmopressin was continued at 60 mcg *nocte* as her DI was persistent after TSS.

Six months after transsphenoidal surgery, the patient has shown clinical improvement with amelioration of her Cushingoid features and resumption of her menses by the 3rd month postoperatively. She has also managed to reduce her weight to 71.1 kg with exercise and diet modification.

DISCUSSION

Our case proved to be interesting as she presented features of CS and central DI which could not be attributed to any detectable stalk lesion on MRI. Most DPA were GH- or PRL-secreting or non-functioning adenomas.[1,6-8] Ogando-Rivas *et al.* demonstrated a higher frequency of GH-secreting adenomas compared with ACTH-secreting adenomas in 17 cases of double or multiple pituitary adenomas identified preoperatively by MRI and confirmed by histology and immunohistochemistry. The age of the patients ranged from 22 to 67 years, and most were female.[9] Other studies also reported a higher frequency in patients with acromegaly, followed by those presenting with Cushing disease.[1,2]

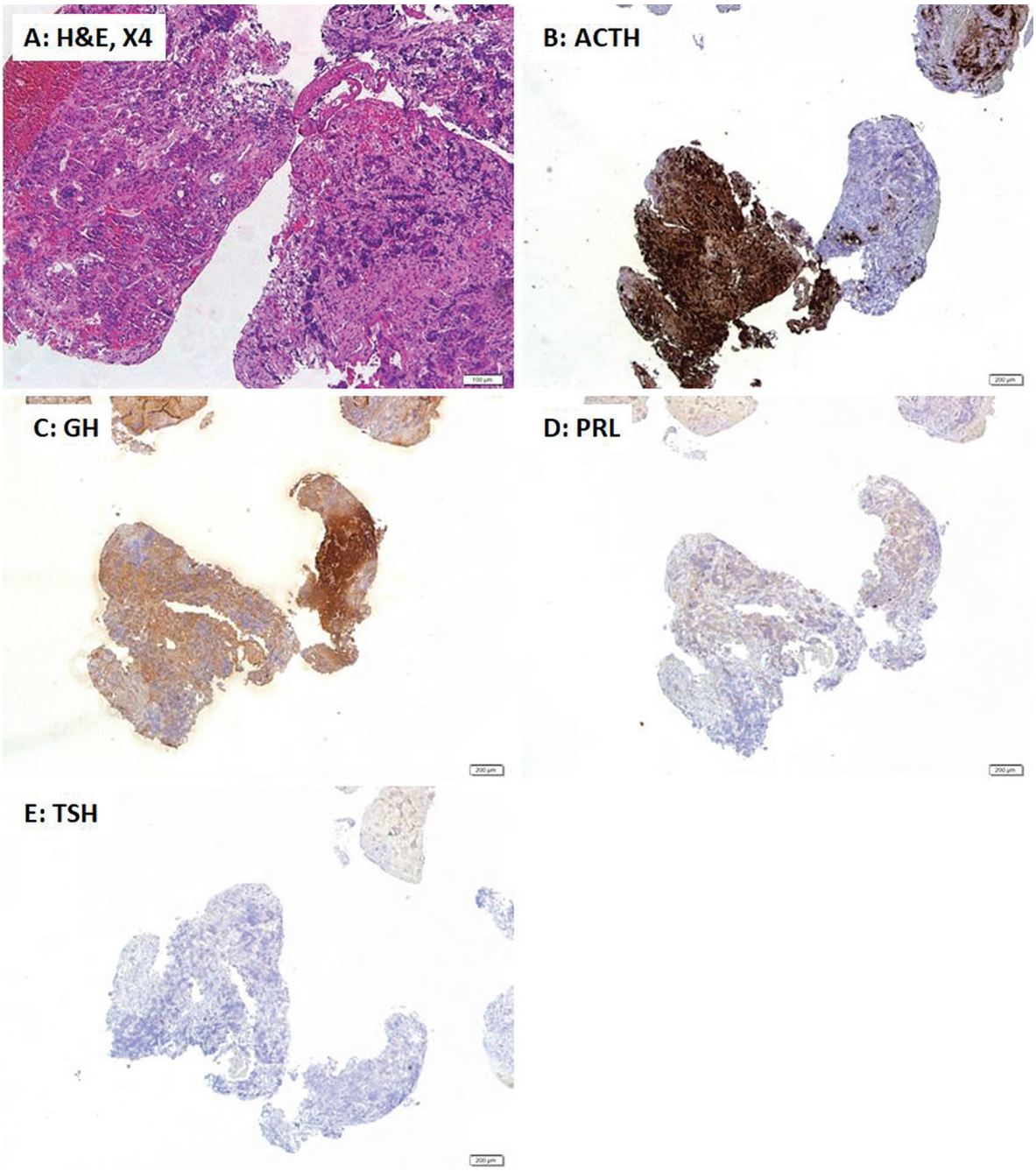

Figure 4. Pluri-hormonal pituitary adenoma located at the midline of pituitary **(A)** (H&E, X4), producing ACTH **(B)**, GH **(C)**, and focally Prolactin **(D)** and TSH **(E)**.

Table 3. Summary of double ACTH pituitary adenomas cases compared to our case

	Year	Age	Gender	Sites of lesion	Immunohistochemistry I & II	Clinical symptoms/ presentation
Case 1 [17]	2010	56	F	Right and left pituitary	ACTH strong & ACTH weak	Cushing disease
Case 2 [18]	2014	38	F	Infundibulum and anterior pituitary	ACTH strong & ACTH strong	Cushing disease
Case 3 [19]	2016	50	F	Right and left pituitary	ACTH strong & ACTH strong	Cushing disease
Case 4 [20]	2017	37	F	Left pituitary lobe	ACTH strong & ACTH strong	Cushing disease
Case 5 [21]	2021	36	F	Right anterior pituitary	ACTH strong & ACTH strong	Cushing disease
Our case	2019	14	F	Right lobe and infundibulum	ACTH strong & ACTH strong	Cushing disease & diabetes insipidus

The incidence of ACTH-secreting tumours with double or multiple pituitary adenomas ranged between 1.6–3.3%.[10-12] In the previous report, 60 cases of multiple pituitary adenomas were observed, of which 58 cases were double adenomas and were grouped according to immunohistochemical criteria. Among all combinations, ACTH- and PRL-secreting tumours seemed to be the most common (33%), followed by GH- and nonfunctional adenomas (24%) and GH-PRL adenomas (10%).[13] Double pituitary adenomas with ACTH hypersecretion have been reported with FSH-secreting lesions, GH-secreting and, most commonly, prolactin-secreting adenomas or silent PRL-immunoreactive adenomas.[1,6,10,14-16] There had been 5 reported cases of double ACTH-secreting pituitary tumours presenting with CS in female patients with an age range of 36-56 years old summarized in Table 3.[17-21] Our case is the youngest and the only patient who manifested Cushing disease with DI. She had two different lesions in the pituitary with strong positive staining for ACTH, GH, LH and TSH, and weakly positive for prolactin and FSH.

It has been reported that the ACTH-secreting adenomas may originate in or extend into the pituitary stalk. Previous literature also observed DPA in both the anterior pituitary gland and stalk. Hence, multiple adenomas should be identified before the operation to achieve curative surgical management.[22] The critical first step in managing double adenomas is their identification, which is based on MRI.[5,12,23] Preoperative MRI is an effective and sensitive method to determine the presence of multiple adenomas.[5,13,24] Pu et al., in their review of 42 patients with CS found that 22 (52.4%) were diagnosed from preoperative MRI, 2 (4.8%) from computerized tomography (CT) scan and the remaining 18 patients were diagnosed during surgery.[21] Previous literature reported MRI to be superior in detecting multiple pituitary adenomas; however, in our patient, the second adenoma could be missed radiologically due to its small size. Hence, intraoperative evaluation via surgical exploration may assist in diagnosing double or multiple pituitary adenomas.[1,9,15,19] Endoscopic TSS is used more often than the microscopic approach, particularly in cases of pituitary lesions.[24] Other literature reviews revealed similar findings with the majority of them being visible to the surgeon by the endoscopic approach.[1,9,15,19]

In our case, the patient underwent neuro-endoscopic TSS, which allowed for better visualization and enabled the detection of another microadenoma which prevented the requirement for a second surgery. Previous cases reported that patients had to undergo two surgeries to resect two pituitary adenomas. Following the first surgery, the disease manifestation and hormone levels revealed that the tumours were not in remission, indicating another microadenoma.[3,12,14] Therefore, distinguishing between a normal pituitary gland and a pituitary adenoma is crucial in surgery.

The only way to ensure that both adenomas are removed surgically is to look at the biochemical and HPE findings. If a single tumour is removed and repeated hormonal work-up shows no evidence of biochemical remission, reexamination is usually required in the early postoperative period. Unfortunately, poor surgical outcomes in patients with DPA have been reported.[7,25] Even if 100% biochemical cure can be achieved, patients are usually left with pituitary insufficiency as demonstrated in our patient who required hormone replacement.[26,27] In the presence of high-resolution MRI scanning and inferior petrosal sinus sampling, a hemi-hypophysectomy can be performed if the patient does not achieve biochemical remission after the surgery to preserve as much glandular tissue as possible. If the lesion is not visible on the pituitary gland surface, then the surgeon can make exploratory incisions into the gland to search for the primary or second tumour to avoid missing the causal adenoma.

CONCLUSION

This is the youngest patient with double ACTH-secreting pituitary adenoma and diabetes insipidus. The coexistence of double adenomas can be challenging to diagnose with poor surgical outcomes. Intraoperative evaluation is important in the identification of double or multiple pituitary adenomas in a patient presenting with multiple secretory manifestations. Post-operative biochemical cure can be achieved but usually ends up with pituitary insufficiency.

Ethical Considerations

The patient's mother has given her consent for the publication of this article.

Statement of Authorship

The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement

WHK: Conception, Curation, Investigation, Writing – original draft preparation; **IIA:** Conception, Curation, Investigation, Writing – original draft preparation; **NAW:** Writing – review and editing.

Author Disclosure
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Data Availability Statement
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CASE REPORT

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Diagnosis and Management of Adrenocortical Carcinoma with Co-secretion of Cortisol and Aldosterone: A Case Report

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Abstract

Adrenocortical carcinoma (ACC) accounts for 0.05-2% of all malignant tumors. Forty-five percent of ACCs with secretory function have excess glucocorticoids alone and only less than 1% secrete aldosterone.

This is a case of a 44-year-old Filipino female with hypertension and a 12-year-history of an incidentaloma of the left adrenal gland, with recent-onset complaints of increasing abdominal girth, purple striae, amenorrhea, moon facies and a dorsocervical fat pad. Laboratory findings revealed low potassium levels, non-suppressed cortisol on dexamethasone test suggesting Cushing's syndrome and elevated aldosterone-renin ratio and plasma aldosterone concentration pointing to primary hyperaldosteronism. A computed tomography scan revealed a left-sided adrenal mass measuring approximately 23 cm in largest diameter suggestive of carcinoma without metastasis or lymph node involvement. Complete resection via open adrenalectomy was performed and histopathologic assessment revealed Adrenocortical Carcinoma with Weiss score of 4. The Ki-67 proliferative index was found to be >20%. Radiotherapy was done as an adjuvant treatment.

Although rare, co-secretion of cortisol and aldosterone can occur in functional tumors of adrenocortical carcinoma. Malignancy should always be considered in patients who present with a history of a unilateral adrenal mass and/ or in those with signs and symptoms of adrenal hormone excess. Thus, a proper assessment derived from a thorough medical history, physical examination and laboratory work-up is warranted in patients with an adrenal mass to ascertain the diagnosis and provide adequate management.

Key words: adrenocortical carcinoma, primary hyperaldosteronism, Cushing's syndrome, cortisol, aldosterone

INTRODUCTION

Adrenal cortical carcinoma (ACC), or adrenocortical carcinoma, is a rare condition with an annual incidence of approximately 1-2 per 1 million of the population and comprises 0.05-0.2% of all cancers.[1] It is highly malignant, often sporadic and linked to mutations in the tumor suppressor gene TP53, alterations in the Wnt/Beta-catenin pathway and overexpression of the insulin-like growth factor 2 (IGF2) cluster.[2] Patients with ACC may be asymptomatic and diagnosed incidentally only during imaging. However, 80% of these tumors are functional and may present with clinical features of adrenal hormone excess. Forty-five percent of these patients have an excess of glucocorticoids alone, 45% with glucocorticoids and androgens and approximately 10% with androgens alone.[3] An evaluation of the adrenal hormone status is warranted, followed by surgical resection of the tumor and adjuvant treatment with mitotane. Monitoring for recurrence is performed indefinitely, with an option for cytotoxic chemotherapy should there be progression or recurrence. This report presents a rare case of cortisol and aldosterone-secreting adrenocortical carcinoma with a clinical presentation of primary hyperaldosteronism and Cushing's syndrome, its diagnosis and management.

CASE

A 44-year-old Filipino female had complaints of increasing abdominal girth with reddish-purple striae on the abdomen and arms. The patient has no history of diabetes or asthma but is a known hypertensive of 6-year duration, with poor control despite 3 different anti-hypertensive maintenance medications. Her family history only revealed hypertension from both paternal and maternal sides, but no known history of malignancies. Twelve years prior, the patient also had a history of an incidental finding on a computed tomography (CT) scan of a left adrenal mass approximately 9 cm in largest diameter. She was advised work up during that time but was not able to comply and was lost to follow-up, thereafter. The patient's present complaint started 2 years prior to admission, wherein

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increasing abdominal girth was associated with gradually increasing abdominal pain, amenorrhea, build-up of a dorsocervical fat pad and a rounded appearance of the face. The condition was tolerated until the time of consult. The initial impression was that of Cushing's syndrome likely due to a functioning adrenal tumor. Laboratory tests taken include thyroid function tests, fasting blood glucose level, glycosylated hemoglobin, lipid profile, a complete blood count, electrolytes, liver and kidney function tests. The patient was found to have impaired fasting glucose and a subnormal potassium level. Correction of the serum potassium was done. An abdominal CT scan with contrast showed a large heterogeneously enhancing, well-circumscribed, noncalcified mass in the region of the left adrenal gland (Figures 1 and 2), measuring approximately 22.5 x 19.6 x 22.8 cm and with no other lesions found in the abdomen. The chest x-ray showed clear lung fields. Positron Emission Tomography (PET) scan did not reveal any evidence of metastasis in other sites of the body.

At this time, mineralocorticoid excess was considered due to the presentation of hypertension, hypokalemia and the presence of an adrenal mass. An overnight dexamethasone suppression test revealed a non-suppressed cortisol at 389.2 nmol/L (14.1 ug/dL) while plasma ACTH was low. These findings along with the clinical signs and symptoms suggested an excess of cortisol and pointed to ACTH-independent Cushing's syndrome. The dehydroepiandrosterone-sulfate (DHEA-S) and 24-hour urine metanephrine tests were within normal range ruling out androgen excess and pheochromocytoma, respectively. The computed aldosterone-renin ratio (ARR) of 33.475 ng/dl:ng/ml/hr indicated an excess of aldosterone. The patient's plasma aldosterone concentration was also increased at 47.20 ng/dL. In patients with a background of hypokalemic hypertension, strongly positive ARR and concurrently increased aldosterone levels, confirmatory testing for primary hyperaldosteronism (like saline infusion test) may not be necessary and can be deferred.[2]

The patient underwent unilateral adrenalectomy via open surgery with peri-operative steroid coverage which yielded a large, grey-tan, firm, ovoid mass that measured 260 x 235 x 155 mm and weighed 3800 grams (see Figure 3). On serial sectioning, the mass showed a predominantly solid, lobulated, yellow-tan cut surface with large hemorrhagic and necrotic areas occupying 40% of the mass. The microscopic examination revealed tumor invasion through the adrenal capsule, low grade with oncocytic features, presence of lympho-vascular invasion and negative margins. The histopathologic findings were consistent with adrenal cortical carcinoma. Post-operatively, chest and lung Positron Emission Tomography and Computed Tomography (PET-CT) scans did not detect metastasis or local recurrence. The patient's blood pressure decreased to normal level and there was no recurrence of hypokalemia. The Ki-67 index, however, had increased expression at >20% on immunostaining, hence, radiotherapy was subsequently done with advice for regular follow-up.

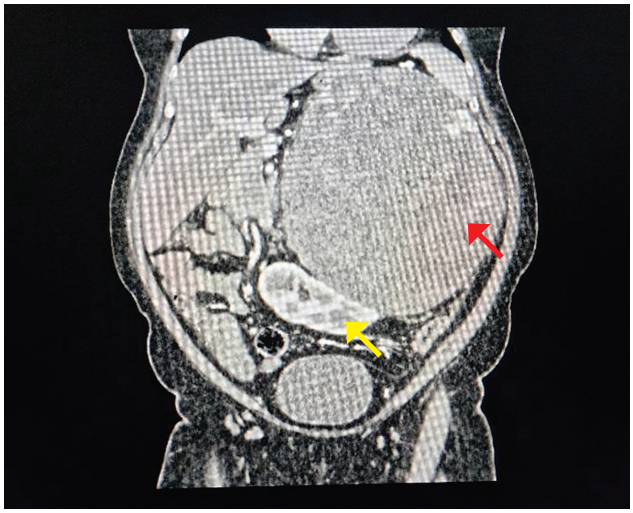

Figure 1. CT scan of the abdomen showing a large heterogeneously-enhancing mass *(red arrow)*, displacing the left kidney *(yellow arrow)* caudally and medially.

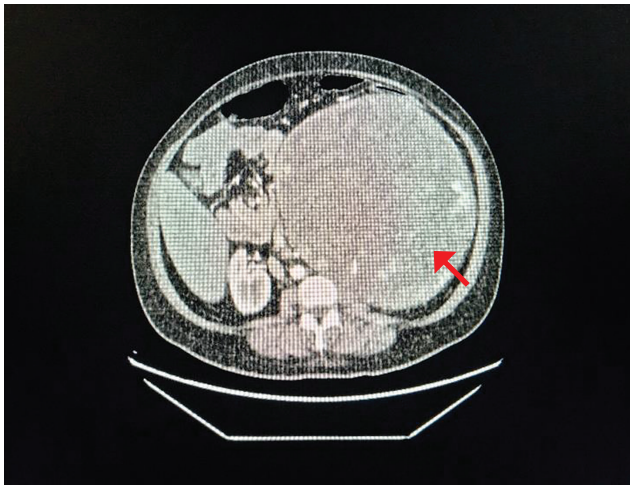

Figure 2. CT scan of the abdomen on axial view showing the left adrenal mass *(red arrow)* occupying the bulk of the left intraabdominal space.

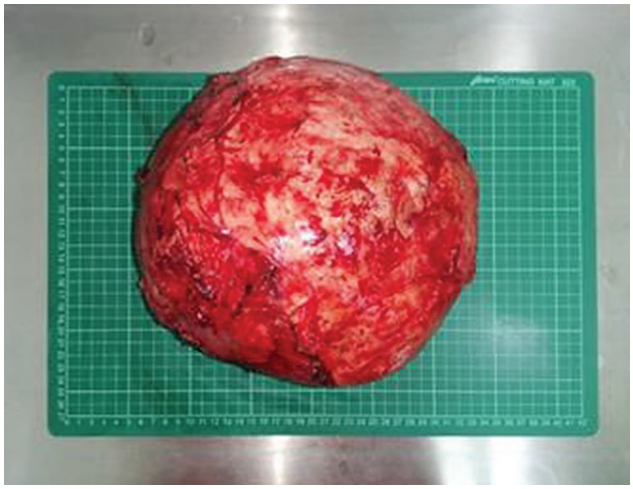

Figure 3. Adrenal mass obtained after surgery, measuring 260 x 235 x 155 mm.

DISCUSSION

ACC is an uncommon underlying cause of unilateral adrenal mass cases, with only an approximate prevalence of 2-5% and 0.05-2% of all malignant tumors.[1] It is a rare malignancy and has an annual incidence of 1 to 2 per million in the population. Women are more affected than men at a ratio of 2.5:1, with a mean age of onset between the 4th to 5th decades of life.[3] The diagnosis of malignancy relies on investigations of clinical, biological and imaging features before surgery and with the subsequent histopathological examination after surgery. Approximately 80% of ACC tumors are functional. Additionally, among the functional tumors, 45% may secrete glucocorticoids alone, 45% may secrete both glucocorticoids and androgens and 10% secrete androgens alone,[3] while only less than 1% of all tumors secrete aldosterone. Thus, the patients would usually present with features of hormone excess. For the case at hand, the patient presented with gradually increasing abdominal pain, increasing abdominal girth, purple striae, amenorrhea, moon facies and a buffalo hump, all of which are clinical manifestations of cortisol hypersecretion from Cushing's syndrome. Furthermore, the patient had a history of a unilateral adrenal mass, uncontrolled hypertension and hypokalemia. These features were suggestive of mineralocorticoid excess which warranted further work-up. As ACC tumors often have an overproduction of glucocorticoids alone or of both glucocorticoids and androgens, the patient's presentation of both glucocorticoid and mineralocorticoid excess was uncommon.

The risk for ACC in a unilateral adrenal mass increases with tumor size, with the index of suspicion increasing for tumors more than 4-6 cm.[3] Adrenal malignancy was suggested by the heterogeneous enhancement and large size of the mass (approximately 23 cm in its largest diameter) with the presence of neovascularization on imaging. Assessment for metastasis was imperative in the patient. Metastasis in ACC most frequently occurs in the liver and lung.[2] Evaluation of the lungs by a chest x-ray is the initial imaging modality used in the detection of pulmonary metastasis. A CT scan of the whole abdomen with contrast media is informative and provides adequate sensitivity for the assessment of the adrenal mass and the detection of hepatic metastasis.[4] Additionally, a PET-CT scan would also provide information on possible metastasis on other sites of the body which may influence therapeutic decision-making.[3] With the imaging procedures done on the patient, other than the visualized adrenal mass, there were no other lesions, lymphadenopathies, or evidence of metastasis found.

Patients with an adrenal mass are worked up for hormone excess. Plasma metanephrine or 24-hour urine metanephrine is measured to assess for catecholamine excess that may indicate the presence of pheochromocytoma.[2] In this patient, plasma metanephrine (normal range <0.5 nmol/L)[3] and 24-hour urine metanephrine (normal range 24-96 mcg/day but may vary in other centers)[3] were within the normal range. In the workup for cortisol excess, screening or confirmatory testing include the following: increased 24-hour urinary free cortisol excretion, failure to suppress morning cortisol level after exposure to dexamethasone overnight and evidence of loss of diurnal cortisol secretion along with high levels at midnight[5] – which is the time at which cortisol secretion is physiologically at its lowest.[3] When results are equivocal, further confirmation may be done by performing a low-dose dexamethasone suppression test.[5] An overnight dexamethasone suppression test was done for the patient in which 1 mg of dexamethasone was given at 11 PM and cortisol level was determined at 8 AM the subsequent day. The patient's cortisol level was found to be elevated and unsuppressed at 389.2 nmol/L which is greater than the cutoff of 50 nmol/L. Plasma ACTH taken was suppressed at 1.04 pg/mL. Low levels of ACTH (<5 pg/mL) would support cortisol excess that is not dependent on ACTH.[2]

In the work-up for mineralocorticoid excess, the clinical suspicion of such is made in the presence of hypertension and at least one risk factor.[2] These risk factors include the presence of an adrenal mass, drug-resistant or severe hypertension needing >3 antihypertensive medications, hypokalemia and family history of early-onset hypertension or cerebrovascular events at <40 years of age. The patient was clinically suspected of mineralocorticoid excess due to poorly controlled hypertension, persistent hypokalemia and the presence of an adrenal mass. Screening is done by measuring aldosterone and renin levels and subsequently assessing the aldosterone-renin ratio. The aldosterone-renin ratio (ARR) is "positive" for mineralocorticoid excess if the ratio is >30 ng/dL per ng/mL/hr with a concurrently high normal or increased aldosterone level.[6] The patient's ARR was noted to be elevated at 33.475 ng/dL:ng/mL/hr and plasma aldosterone concentration was high at 47.20 ng/dL. Normal levels of plasma aldosterone concentration range from 7 to 30 ng/dL.[6] The second step in the assessment for mineralocorticoid excess is to do confirmatory testing to show that the excess in aldosterone is produced autonomously or independently of the renin-angiotensin system. However, confirmatory testing may be omitted when any of the following factors are present: elevation of the plasma aldosterone concentration >20 ng/dL, presence of hypokalemia and/or undetectable plasma renin activity.[6] The patient presented with hypokalemic hypertension and had a plasma aldosterone concentration of 47.20 ng/dL, thus, confirmatory testing was deferred. Patients with primary hyperaldosteronism generally should undergo adrenal vein sampling (AVS) to distinguish between unilateral or bilateral aldosterone excess.[3] This, however, may not be done in patients suspected of having an adrenocortical carcinoma.[7]

Evaluation of overproduction of adrenal androgen precursors should also be done in the context of an adrenal mass with the potential of androgen excess or potential adrenocortical cancer. Elevation of dehydroepiandrosterone-sulfate (DHEAS) may be frequently seen in the context of

ACC.[2] Hence, DHEAS levels were taken and were within normal range in this patient. Reference ranges for DHEAS differ based on age and sex, but a 44-year-old female generally has normal DHEAS levels between 57.3 and 279.2 mcg/dL.[8]

Staging in ACC is mandatory to assess for prognosis and treatment options. The TNM staging system is recommended and defines Stage I and Stage II as strictly localized tumors that differ in size at ≤5 or >5 cm, respectively. Stage III ACC is characterized by infiltration of the surrounding tissue, the presence of positive regional lymph nodes, or a tumor thrombus in the vena cava and/ or renal vein, whereas Stage IV ACC differs from the rest by the presence of distant metastasis. Stages I-III are considered localized ACC and complete resection by surgery is the treatment of choice.[4] As the patient's mass was well-circumscribed and did not have evidence of regional lymph node involvement, infiltration of the surrounding tissue, or distant metastasis on imaging procedures, the case was assessed to be that of Stage II. Open surgery is the standard surgical approach for patients with confirmed or highly suspected ACC.[9] Complete resection of the adrenal tumor by open adrenalectomy was, therefore, performed.

Following surgery, pathological assessment of the specimen was performed to verify the diagnosis, evaluate prognostic markers and assess the need for adjuvant therapy.[10] Macroscopically, ACC tumors are large and heterogeneous, with a surface that ranges from brown to orange or yellow, depending on the lipid content of their cells. Necrosis is almost always present. Gross examination of the specimen revealed a large, slightly firm, ovoid mass measuring 26.0 x 23.5 x 15.5 cm and weighing 3.8 kilograms. It presented with a smooth, glistening, grey-tan external surface, and serial sectioning revealed a predominantly solid cut surface with hemorrhagic and necrotic areas occupying approximately 40% of the entire mass. On microscopic examination, the evaluation of the specimen makes use of the Weiss score, which is the best-validated score for establishing ACC. It takes into account the presence of necrosis, invasion of various structures, diffuse architecture, atypical mitosis and the nuclear grade. A score of ≥3 defines ACC, while a score of 1-2 supports adrenal adenoma.[4] The presence of necrosis, atypical mitotic figures, capsular invasion and lympho-vascular invasion in the patient's evaluated specimen, favor the diagnosis of carcinoma with a Weiss score of 4. Immunohistochemical staining for the Ki-67 proliferation index helps to define the prognosis of ACC. A Ki-67 <10% is indicative of slow to moderate growth velocity, whereas a Ki-67 of ≥10% is associated with poor prognosis and a high risk of recurrence and rapid progression.[1] The immunohistochemical staining revealed a Ki-67 of >20% in the patient. Due to the high risk of recurrence, patients with a high proliferation index are offered adjuvant therapy with mitotane and/ or radiotherapy.[4] After an individualized discussion with the patient and taking into account her risk factors and potential adverse effects, a decision was made to perform radiotherapy. Although there is ongoing debate regarding the subset of patients that are recommended radiotherapy, evidence suggests a potential benefit of this treatment in all stages of ACC, and it is more commonly recommended for patients with a high risk of recurrence or those with Stage 3 disease.[10]

CONCLUSION

A thorough history and physical examination along with a comprehensive laboratory work-up is warranted in patients with an adrenal mass to ascertain the diagnosis and provide adequate management. Although rare, adrenocortical carcinoma can be the primary cause of an adrenal mass, and although largely uncommon, it can cause hypersecretion of both aldosterone and cortisol that would, therefore, present with clinical features associated with the excess of these hormones. Uncontrolled hypertension and hypokalemia are often indicators of primary hyperaldosteronism on a background of an adrenal mass. On the other hand, the clinical manifestations of Cushing's syndrome would highlight an excess of cortisol. These include, but are not limited to, the presence of a buffalo hump or the formation of a dorsocervical fat pad, moon facies, abdominal striae and weight gain. The presence of an adrenal mass most often points to an adrenal adenoma; however, certain features favor the presence of an adrenal carcinoma. These features include the large size of the adrenal mass greater than 4-6 cm in diameter, the heterogeneous character of the mass on imaging especially at >20 HU and invasion to surrounding structures. The presentation of some or all of these features should, therefore, highlight primary adrenal malignancy as a possible cause. Complete surgical resection is the standard of care in patients with adrenocortical carcinoma. Determination of the Ki-67 proliferation index is a prognostic marker that would then guide post-operative treatment. Adjuvant therapy with mitotane and/ or radiotherapy is done, taking into account the patient's risk factors and prognostic markers, balanced with the likely adverse effects of treatment. Regardless of the prognosis and treatment, lifelong monitoring for recurrence is recommended.

Ethical Consideration
Patient consent was obtained before submission of the manuscript.

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

CRediT Author Statement
MMA: Conceptualization, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration; **LMTG:** Conceptualization, Investigation, Writing – original draft preparation, Writing – review and editing

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CASE REPORT

A Focal Form of Diazoxide-resistant Congenital Hyperinsulinism with Good Response to Long-acting Somatostatin

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Abstract

A four-year-old female who was born term via spontaneous vaginal delivery (SVD) with a birth weight of 3.4 kg had an onset of persistent hypoglycaemia at the 6th hour of life. She was diagnosed with congenital hyperinsulinism based on high glucose load, negative ketone and a good response to glucagon. Genetic workup revealed the presence of ATP Binding Cassette Subfamily C Member 8 (ABCC8 genes) mutation which indicated a focal form of congenital hyperinsulinism. She was resistant to the standard dose of oral diazoxide but responded to subcutaneous somatostatin. At the age of 3 years and 6 months, multiple daily injections of somatostatin were replaced with a long-acting monthly somatostatin analogue. With the present treatment, she had better glycaemic control, normal growth and was able to stop tube feeding.

Key words: congenital hyperinsulinism, hyperinsulinaemic hypoglycaemia, focal congenital hyperinsulinism, somatostatin

INTRODUCTION

Congenital hyperinsulinism (CHI) is the leading cause of persistent or refractory hypoglycaemia in the neonatal period. It is due to mutation in the genes that regulate insulin secretion by the ß-cells.[1] Fourteen (14) key genes are responsible for CHI. The most common genetic mutation is the mutation in K_{ATP} channel which is made up of 4 subunits encoded by Potassium Voltage-Gated Channel Subfamily J (KCNJ11) or ATP Binding Cassette Subfamily C Member 8 (ABCC8 genes).[2] However, only about 40-50% have known genetic mutations and majority of the putative genes have yet to be discovered. Histologically, CHI is classified into either diffuse pancreatic ß -cell hyperplasia characterised by diffuse involvement of pancreatic ß -cells or focal pancreatic ß-cells hyperplasia with only focal ß-cell hyperplasia. Familial forms of CHI can exhibit recessive or autosomal dominant patterns of inheritance while the sporadic form is very rare, accounting for about 1 in 30-50000 live births.[3]

Clinically, CHI can be classified according to treatment response to diazoxide which can be either diazoxide sensitive or resistant.[4] Diazoxide is the gold standard of treatment for CHI which acts by binding to sulphonlyurea receptor 1 (SUR1) on the K_{ATP} channel to suppress insulin release by ß- cells. The initial dose is 5 mg/kg/day given in 3 divided doses up to 15-20 mg/kg/day. The most severe side effects are fluid retention, cardiac failure, and pulmonary hypertension.[5] Hydrochlorothiazide at 7-10 mg/kg/day given in 2 divided doses, is usually added to diazoxide to prevent fluid retention. Glucagon is recommended in the acute management of persistent hypoglycaemia, and it is used short-term as a key counterregulatory hormone to oppose the effect of excess insulin. Nifedipine is another first-line medication which acts as a calcium channel blocker to prevent calcium influx leading to insulin exocytosis. The recommended dose is 0.25-2.5 mg/kg/day divided into 2-3 doses, however, it is not a preferred choice because of associated hypotension with a dose that exceeds 0.5 mg/kg/day.[5] Octreotide is used as a second-line medication which is reserved for patients who fail to respond to first-line pharmacological intervention. It is a polypeptide chain with eight amino acids that suppresses insulin release by binding predominantly to somatostatin receptors (SSTRs) 2 and 5. The recommended initial dose is 5 μg/kg/day given by either subcutaneous injection every 6-8 hours or continuous infusion with a maximal dose of 30-35 μg/kg/day. The first response to octreotide is hyperglycaemia followed by a blunted effect after 48 hours (tachyphylaxis).[5] Other potential medications are sirolimus, exendin and glucagon analogue. Sirolimus is an immunosuppressive agent with an anti-proliferative ability which inhibits the mammalian target of rapamycin (mTOR). Its mechanism of action in CHI has not been fully elucidated. The reported side effects are mainly due to immunosuppression and more trials are needed to determine its safety and efficacy in CHI.[6] Exendin is glucagon-like peptide receptor antagonist that works by reducing cyclic adenosine monophosphate (cAMP) to suppress insulin release. It is a potential medication to prevent fasting hypoglycaemia and protein-induced

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hypoglycaemia, but future trials are needed to evaluate its safety and efficacy.[7] Glucagon for long-term usage is hampered by its poor solubility and stability. A glucagon analogue has been developed in an animal model that may provide potential weekly treatment for CHI.[8]

The standard treatment for the focal form of CHI is surgical resection of the focal site in the pancreas that results in cure of CHI.[9] Pancreatic surgery for CHI should only be performed in established CHI centres that could perform an 18-F DOPA PET scan to localise the lesion. Due to the complexity of the pancreatic surgery and logistic factors, most of the diazoxide-resistant CHI are treated with short-acting standard octreotide injection that requires multiple injections in a day. We share our experience in the transition of standard octreotide to monthly long-acting somatostatin in a patient with focal CHI.

CASE

This is a case of a 4-year-old female who is the youngest of 2 siblings from a non-consanguineous marriage. Her mother had no history of gestational diabetes. She was born via spontaneous vaginal delivery at 39 weeks age of gestation, with a birth weight of 3.4 kg. Her APGAR score was 9 at 5 minutes, and 10 at 15 minutes. At the 6th hour of life, she was found to be inactive, pale and feeding poorly. Capillary blood glucose was 1.6 mmol/L. An intravenous bolus of D10% 2 ml/kg was administered and the patient was admitted to the intensive care unit for further management. She required multiple dextrose D10% boluses in increasing frequency to maintain a blood sugar level of more than 2.6 mmol/L. No dysmorphism, cleft lip/palate or neurocutaneous signs were observed. Other systemic examinations were unremarkable. Her plasma glucose level was noted to be more stable at about 2.6-3.3 mmol/L only at a glucose infusion rate (GIR) of 15 mg/kg/min, glucagon infusion at 10 mcg/kg/min, and hydrocortisone given at 1mg/kg body weight thrice a day at about 48 hours of life. During an episode of hypoglycaemia, her urine ketone was negative, c-peptide 0.5 nmol/L, lactate 1.0 mmol/L. The patient was also empirically started on intravenous crystalline penicillin/gentamicin to cover for neonatal sepsis. With the high GIR, negative ketone, good response to glucagon and presence of insulin during hypoglycaemia, she was treated as a case of persistent hypoglycaemia secondary to congenital hyperinsulinism (CHI) or persistent hypoglycaemic hyperinsulinaemic of infancy (PHHI). Oral diazoxide was started at an initial dose of 2 mg/kg/day in 3 divided doses and the dose was increased every 2 days guided by blood sugar trends. Despite being on a high dose of oral diazoxide at 20 mg/kg/day, the patient continued to have intermittent hypoglycaemia, which raised suspicion of the presence of a diazoxide resistance form of CHI. Octreotide at a dose of 8.8 mcg/kg/day was started, and the patient showed a remarkable improvement in the blood sugar pattern. DNA from the patient and both her parents were extracted and sent to Exeter Lab UK for DNA sequencing. She was discharged home at 62 days of life with subcutaneous octreotide injection (10 mcg/kg/day) in divided doses and was advised for home blood glucose monitoring. She was on-demand breastfeeding and infant formula via a feeding tube every 2 hours. Weaning was started at 6 months of life with porridge, biscuits, and bread. She was a picky eater and growth was observed to be at the 3rd- 25th percentile for length and weight in the first 6-7 months of life (Figure 1). In the first year of life, she had 3 hospital admissions for acute gastroenteritis, bronchopneumonia, and viral infection at the age of 3 months, 6 months, and 7 months, respectively. She had more hypoglycaemic episodes whenever she was unwell during hospital admissions. Her octreotide dose was increased to 12 mcg/kg/day to counteract hypoglycaemic episodes. In the first 2 years of life, HbA1C ranged from 3.8 to 5.0 % with an average of 4.5%. DNA sequencing revealed that she is heterozygous for a paternally inherited pathogenic *ABCC8* frameshift variant. Her father has the same mutation, but her mother was negative for the mutation. The genetic variant is

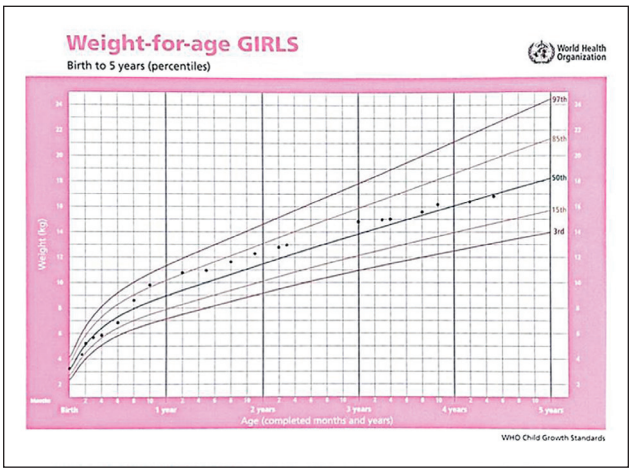

Figure 1. (A) Girls chart: weight-for-age GIRLS [birth to 5 years (percentile).

https://cdn.who.int/media/docs/default-source/child-growth/child-growth-standards/indicators/weight-for-age/cht-wfa-girls-p-0-5.pdf?sfvrsn=d4a8e3bc_12.

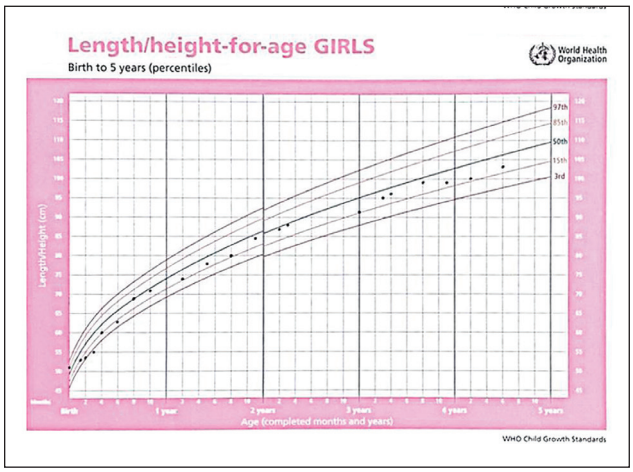

Figure 1. (B) Girls chart: length/height-f-r-age GIRLS [birth to 5 years (percentile)].

https://cdn.who.int/media/docs/default-source/child-growth/child-growth-standards/indicators/length-height-for-age/cht-lhfa-girls-p-0-5.pdf?sfvrsn=d15415dc_10.

highly correlated with a focal form of CHI. The pancreatic focal lesion should be confirmed with an 18F-DOPA PET scan and pancreatic surgery is recommended to remove the focal lesion and attain a cure of the disease.[10] However, due to the unavailability of the 18-F Dopa and experienced surgeon in CHI, the diagnostic scan and surgery were not possible. Intensive medical therapy with regular tube feeding and somatostatin/octreotide were continued. Neurodevelopmental milestones were fairly on time, with a bit of speech delay. She was able to walk at the age of 12 months, drink with a cup at 11-12 months, and speak in 2-3 word sentences at the age of 3 years old. An intramuscular 10 mg long-acting octreotide/Sandostatin LAR was started monthly in place of subcutaneous short-acting octreotide at the age of 3 years and 6 months old. Subcutaneous octreotide was gradually withdrawn over a period of 3-4 weeks. The patient was able to tolerate monthly injection of Sandostatin. HbA1C was 4.8% with a range of 4.6 to 4.9%. She had no liver transaminitis, IGF-1 was normal for age and there was no gall bladder sludge detected on ultrasound. After about few months on long-acting somatostatin, her parents managed to stop tube feeding and she no longer had food aversion.

DISCUSSION

Somatostatin is a peptide/somatotroph release-inhibiting factor that acts primarily via five transmembrane G-protein coupled SST receptor (SSTR 1-5). SSTR5 is highly expressed in beta cells. SSTR share common signalling pathways such as the inhibition of adenyl cyclase, activation of phosphotyrosine phosphatase and modulation of mitogen-activated protein kinase through a G-protein dependent mechanism. SSTR 2-5 are coupled to the inward rectifying K channel. The binding of SST to SSTR on the beta cells results in the inhibition of voltage-gated calcium channels and reduces cAMP levels thereby inhibiting insulin release.[11]

There are two formulations of octreotide/Sandostatin, the regular form and octreotide LAR/long-acting form. Octreotide is an octapeptide with 4 amino acid sequences essential for biological activity. The incorporation of N-phenylalanine, L-terminal amino-alcohol, D-tryptophan and cysteine bridge make them more resistant to peptidase degradation compared to its natural form that has a very short half-life of 3-5 minutes.[12]

The prevalence of CHI is 1 in 50000 live births. Most individuals with CHI respond to first-line treatment with diazoxide and only about 40% are resistant. Eighty to ninety percent of diazoxide-resistant CHI have a mutation in the K_{ATP} channel. Pancreatic ß -cells express K_{ATP} channels that are needed for normal insulin secretion and are targets for drugs that modulate insulin secretion. This is made up of 4 subunits encoded by *KCNJ11* or *ABCC8 genes*. Inactivating mutations in the genes encoding the two subunits of the ATP-sensitive potassium cause diazoxide-resistant CHI.[5] Long-acting SST has been used for the treatment of diazoxide-resistant CHI secondary to diffuse form since surgery for diffuse form carries more short-term and long-term complications such as persistent hypoglycaemia, biliary tree injury, pancreatic fistula, bleeding, hepatic/splenic injury, diabetes, and pancreatic endocrine/exocrine dysfunction.[13] There are many published studies on the use of long-acting SST in the diffuse form of CHI which showed that it is safe, effective and resulted in improved quality of life. Shah et al., reported an improvement in the quality of life for a patient who experienced severe side effects associated with diazoxide while Novokreshhennyx et al., reported that 67% achieved euglycaemia with a therapeutic dose of lanreotide given at 3.5-5.5 mg/kg/month, without any significant side effects.[12] Ivo van der Steen et al., reported 89 % improvement in blood glucose control without any life-threatening side effects while 37% experienced transient elevation of liver enzymes with the use of long-acting SST.[5]

There are not many publications on the use of long-acting somatostatin in the focal form of CHI. The efficacy of long-acting SST is believed to be related to the expression of SSTR2 in focal pancreatic tissues which would shut off the excessive insulin production after binding to SST.[14] The use of long-acting somatostatin in the focal form has been demonstrated to be safe and effective in a very small number of patients from case reports since the focal form is very rare and a majority of patients were on standard octreotide treatment, and some underwent surgery.[15] It was also used for patients who had failed pancreatic surgery with post-operative hypoglycaemia, and for patients who refused surgical intervention. There were no life-threatening side effects associated with the use of long-acting somatostatin in the focal form of CHI. Our patient is a confirmed case of a focal form of CHI secondary to *ABCC8* gene mutation. An 18F-DOPA PET scan is not available in many countries including Malaysia. To our knowledge, it is only available in certain European countries, the United States and Japan due to the short half-life of radioactive material used in PET scans to localise the focal lesion in CHI. Furthermore, pancreatic surgery for CHI should only be performed in a CHI centre with a high patient load. Our patient has been on long-acting SST for 13 months whereas the patient in the study of Pratik et al., was on the treatment for 7-27 months.[16] We used a brand named Sandostatin since it is easier to obtain compared to lanreotide. Both are SST analogues that are reserved for the treatment of neuroendocrine tumour in adults but in the paediatric population, they are used as an off-label medication for CHI.

There are several complications secondary to SST treatment such as necrotising enterocolitis, abdominal distension, fat malabsorption, gall bladder sludge/stone, liver transaminitis, hypothyroidism and growth impairment. Transient transaminitis and asymptomatic gall bladder sludge are the most prevalent complications.[17] Van der Steen et al., found that 13 out of 27 subjects (48%) had mainly mild to moderate side effects such as pain at the injection site, nausea, vomiting, and diarrhoea. Long-term treatment with SST has been associated with gall bladder stone formation, cholestatic jaundice, and elevated liver enzymes that are reversible after stopping treatment.[18] It is recommended to regularly screen for the side effects secondary to long-term use of octreotide in children. For our patient, she had

a transient increase in liver enzymes with a high dose of SST at the age of about 1-2 years old. The side effects were dose-dependent and later on, the liver enzymes normalised with the reduction of SST doses, optimization of enteral feeding and transition to long-acting SST.

CONCLUSION

The case illustrates that focal CHI may be managed by administering monthly long-acting SST. The use of long-acting SST could result in avoidance of pancreatic surgery which may be associated with short- and long-term complications. It is a good alternative for countries without access to 18F-DOPA PET scans or a high-volume surgeon. This conservative approach seems to achieve acceptable glycaemic control and a better quality of life. It is less stressful to the patient and parents since the injection is only monthly. The side effects are less compared to oral diazoxide and subcutaneous octreotide that require multiple injections a day. This approach would also be more popular since CHI becomes less severe as the patient gets older due to the gradual increase in the rate of focal β-cell apoptosis.[19]

Ethical Consideration
Patient consent was obtained before submission of the manuscript.

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

CRediT Author Statement
SH: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project Administration, Funding acquisition; **NSMF:** Resources; **SF:** Investigation

Author Disclosure
The authors declared no conflict of interest.

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CASE REPORT

A Case of Osteitis Fibrosa Cystica of the Mandible: A Rare Presentation during Pregnancy due to *CDC73* Mutation

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Abstract

Typically, primary hyperparathyroidism (PHPT) develops as a result of multiglandular hyperplasia, parathyroid cancer, or parathyroid adenoma. Patients usually present with skeletal manifestations such as low-trauma fractures. Osteitis fibrosa cystica (OFC) is a classic yet rare skeletal manifestation of advanced PHPT currently reported in less than 2% of patients. We present a case of a 29-year-old Indian female who presented with a femur fracture and mandibular OFC 20 days after delivery. The painless mandibular swelling gradually progressed from the third month of pregnancy. The biochemical and radiological investigations were indicative of PHPT-associated OFC. After the excision of the three-and-a-half parathyroid gland, histology revealed benign cystic adenomas and hyperplasia. Based on the associated clinical manifestations, OFC was suspected. Clinical exome sequencing revealed *CDC73*(+) c.687_688dupAG heterogenous pathogenic autosomal dominant mutation. Undiagnosed PHPT in mothers during pregnancy led to neonatal hypocalcaemic convulsions. With adequate supplementation, the infant recovered completely from transient congenital hypoparathyroidism. OFC is an important diagnosis to consider in a young patient with swelling of the neck and jaw. Simultaneous high levels of PTH and serum calcium should raise a high index of suspicion for OFC. Parathyroidectomy helps manage the biochemical abnormalities and causes regression of the jaw mass that causes facial disfigurement and attenuates the declining BMD. Children born to mothers with PHPT should be evaluated for neonatal hypoparathyroidism and supplemented appropriately to reduce the risk of hypocalcaemic manifestations that can be life-threatening. If the *CDC73* mutation is detected, the offspring should be monitored for signs of PHPT due to the high probability of inheritance and parathyroid malignancy.

Key words: osteitis fibrosa cystica, primary hyperparathyroidism, pathological fracture, skeletal manifestation

INTRODUCTION

Primary hyperparathyroidism (PHPT) is a common endocrine disorder with a wide range of estimated prevalence (0.4 to 21.6 cases per 100,000 person-years), probably due to variations in screening globally.[1] However, PHPT is rare in pregnancy, with a reported incidence of 1%.[2] The association of PHPT with 'osteitis fibrosa cystica' (OFC), also known as von Recklinghausen disease, was established in 1925.[3] Osteitis fibrosa cystica is a classic yet rare skeletal manifestation of advanced PHPT currently reported in less than 2% of patients.[4,5] Usually, OFC may be found in any part of the skeleton but it is commonly seen in ribs, clavicle, and pelvis.[6] However, OFCs have a very low incidence (0.1%) in jaws.[6,7] Besides, 90% of the reported cases of OFC occur in hyperparathyroidism due to parathyroid carcinoma, with very few cases attributed to benign growths.[8] Osteitis fibrosa cystica largely occurs in females in the fourth or fifth decade of life; therefore, hereditary patterns should be suspected in younger patients. Hyperparathyroidism has been reported to be an independent feature of the persistent germline tumor suppressor gene (*CDC73*) mutation.[9]

In this case report, we elaborated on the case of a young, pregnant woman who developed PHPT due to parathyroid adenoma associated with *CDC73* mutation, which initially manifested as an OFC of the jaw and subsequently as a femur fracture. The neonate also developed hypoparathyroidism and consequent hypocalcaemia.

CASE

A 29-year-old female, 20 days after delivery, was referred to our facility for a confirmed femur fracture on radiograph accompanied by painless, progressive anterior neck and lower jaw swelling.

Approximately seven months before referral to our hospital, the patient had noticed painless anterior neck

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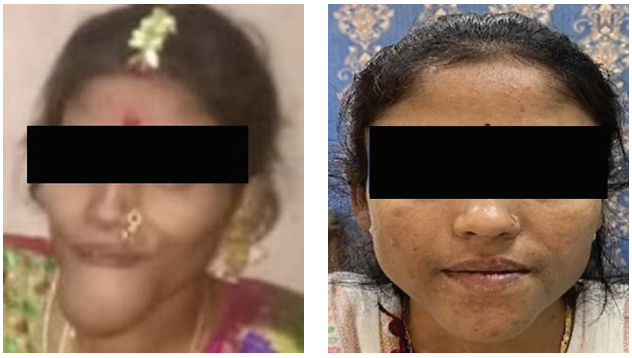

Figure 1. (A) Pre-operative anterior neck and jaw swelling; **(B)** post-operative resolution of the jaw and neck swelling.

swelling and gradually increasing jaw swelling (Figure 1A), on the third month of gestation. Around the seventh month of gestation, a local dentist evaluated the jaw and neck swelling (Figure 2A). An X-ray revealed a solitary expansile lesion of the central body of the mandible spread across the teeth. It had a distinct sclerotic rim, but no resorption of dental roots was noted. Ultrasonography of the neck revealed a cystic lesion in the right thyroid lobe, 43 x 31 mm, most likely a colloid cyst, and a well-defined mass lesion of 51 x 37 mm of the mandible. There was substantial vascularity in the premental, paramental, and submental regions. Subsequent fine needle aspiration cytology (FNAC) of the mandibular lesion revealed multinucleate giant cells or osteoclastomas. She was advised conservative management until delivery due to risks associated with general anesthesia.[10] She also developed right hip pain, which led to a limping gait. The patient ascribed the pain to the pregnancy; hence, avoided seeking medical advice. She delivered a baby boy at term by lower segment cesarean section (LSCS). The baby's birth weight was 2.2 kg, with an Apgar score of 10/10 and he cried immediately after birth. The cry, tone and activity of the neonate were normal. The neonate was exclusively breastfed successfully and passed stools daily until day 21 of life.

Within 12 hours after delivery, the patient developed an altered sensorium with irrelevant speech and was transferred to the Medicine Intensive Care Unit (MICU). On examination, her pulse was 112 beats per minute, blood pressure 112/70 mm Hg and blood glucose 86 mg/dL. Fundus examination was normal. Central nervous system examination suggested delirium without focal neurological deficits. Further investigations in the MICU revealed only an abnormal alkaline phosphatase [20.37 µkat/L reference range 0.63- 2.10 µkat/L] and low serum potassium [3.30 mmol/L (reference range 3.5-5.5 mmol/L)]. Serum calcium was not assessed. Magnetic resonance imaging (MRI) of the brain with MRV showed symmetric hyperintense signals in the bilateral basal ganglia and the midbrain. These findings were considered to be possibly associated with either acute hypoxic insult or metabolic disorder by the treating physician. Metabolic encephalopathy due to hypercalcemia was not suspected by the treating physician. The patient improved with supportive treatment and was discharged after 72 hours.

On day 20 post-partum, she was further evaluated for worsening right hip pain associated with difficulty walking and limping. A hip radiograph revealed a fracture of the right femur (Figure 2B). She was referred to the endocrine service at a tertiary care facility because of a low-trauma fracture at a young age. This prompted laboratory investigations which showed a high serum total calcium at 3.33 mmol/L (reference 2.10-2.55 mmol/L), alkaline phosphatase 5.73 µkat/L (reference 0.63-2.10 µkat/L), parathyroid hormone 684 ng/L (reference 15-68 ng/L) and low serum 25-hydroxyvitamin D 22.91 nnmol/L (reference 74.88-249.60 ng/mL) (Table 1). Thus, after nearly 8 months since symptom onset, PHPT was diagnosed. A subsequent sesta methoxy isobutyl isonitrile (MIBI) scan revealed a parathyroid adenoma at the lower pole of the right thyroid lobe (Figure 2C). Therefore, surgical intervention was sought, and a right inferior parathyroidectomy was performed in a hospital with limited facilities. Intraoperative monitoring of PTH and intraoperative frozen section facilities to predict the postoperative level of PTH were unavailable at the hospital. However, the patient was unwilling to travel to a larger tertiary care centre due to her child's young age. Histopathological examination also revealed benign parathyroid cystic adenoma and hyperplasia. After surgery, there was a decrease in serum

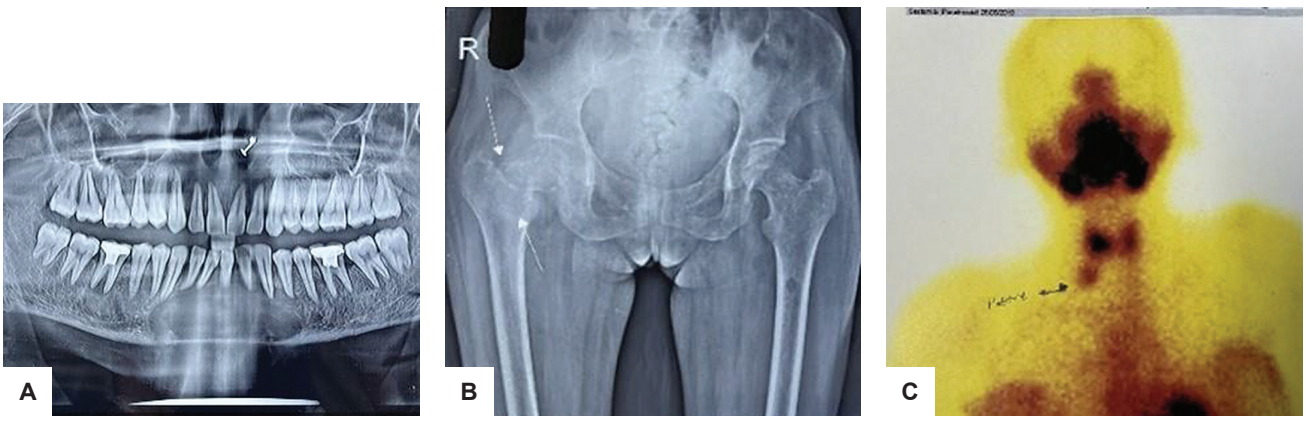

Figure 2. Radiological imaging of the **(A)** jaw, **(B)** femur neck fracture and **(C)** Sestamibi of the right inferior parathyroid adenoma.

Table 1. Laboratory investigations of the patient (Mother)

Test Reference range Unit	Total Calcium 2.10-2.55 mmol/L	Phosphorus 0.81- 1.45 mmol/L	Alkaline phosphatase 0.63-2.10 µkat/L	25-OH Vitamin D_3 74.88-249.60 nmol/ml	Intact PTH 15-68 ng/L
Days post-partum (day concerning surgery)					
1 d	not done†	not done†	20.37	not done†	not done†
22 d	3.33	0.52	5.73	22.91	684
27 d (One day before surgery)	3.05	0.50	6.1	22.1	NA
28 d (Immediately after surgery)	2.63	0.55	6.23	NA	NA
33 d/1 mo, 2 d	2	0.61	6.4	NA	NA
61 d/ 2 mo	2.5	1.07	6.2	84	184
72 d/2 mo, 11d	2.55	0.81	6.75	50.17	115
105 d/3 mo,13 d	2.20	0.94	6.30	144.77	225
124 d/4 mo	2.30	1.20	11.16	126	209
142 d/4 mo 20d (One day before second surgery)	2.35	1.55	10.39	156	280
153 d/5 mo (11 days after second surgery)	2.42	0.84	4.91	137.28	120
213 d/7 mo	1.12	1.36	3.22	86.61	94
615 d/1 y, 8mo, 4 d	2.45	1.02	2.9	95	56
704 d/1 y 11 mo 4 d	2.42	0.95	2.3	50.67	37
1184 d/3 y 2 mo 27 d	2.42	0.91	1.9	12.30	45

† HPT diagnosis not suspected – hence tests not done; d, day; mo, month; NA, not available; y, year

calcium (from 3.3 to 2.10 mmol/L) and iPTH (from 684 to 184 ng/L), though the latter was not maintained within the normal range. Besides, no significant improvement occurred in the hip pain or mandibular lesion.

Approximately two months after the first solitary parathyroidectomy, a progressive increase in serum calcium (2.55 mmol/L) and PTH (225 ng/L) levels were noted in follow-up investigations. On evaluation for persistent PHPT, she was found to have a low MIBI avidity in proximity to the inferior pole of the left lobe of the thyroid on sestamibi scan and arterially enhancing soft tissue lesion in the left paratracheal region on 4D CT neck suggestive of parathyroid adenoma. A dual-energy X-ray absorptiometry (DXA) scan of the femur, radius ulna, and spine showed a t score of -4.1, -5.3, and -4.8, respectively, which confirmed the diagnosis of osteoporosis.

Table 2. Parathyroid levels pre- and post-parathyroidectomy

Event	PTH
Pre-operative Serum PTH	321 pg/ml
First gland removal: Left inferior parathyroid	284 pg/ml
Left superior parathyroid gland removal	213 / 167 pg/ml
Rt superior half parathyroid gland removal	112 / 78 pg/ml

PTH, parathyroid hormone

She underwent a left inferior parathyroid excision at a tertiary care facility equipped for parathyroid surgery. Intraoperative frozen section revealed parathyroid hyperplasia. Nevertheless, no significant decline in intraoperative PTH level was noted; hence, further exploration was conducted (Table 2). Hyperplasia in the left superior and right superior parathyroid glands was confirmed in the frozen section. Therefore, full left superior, full left inferior and half right superior glands were excised, leaving a remnant half-right superior gland. The histopathological analysis was consistent with benign parathyroid hyperplasia. Hungry bone syndrome, commonly occurring as a post-parathyroidectomy complication, was managed with intravenous calcium infusion followed by oral calcitriol, calcium and magnesium supplementation. A year after the three-and-a-half parathyroidectomies, the serum calcium (2.42 mmol/L) and intact PTH (45 ng/L) have been restored to normal levels without calcium and 25-OH vitamin D_3 supplements. She also has completely recovered from bone pain, and the OFC of the jaw has significantly regressed (Figure 1B). The timeline of events is depicted in Figure 3.

Given the young age, presence of parathyroid adenoma and OFC of the jaw, genetic studies were recommended.

Table 3. Clinical profile and laboratory investigations of the child

Chronological age	Body Weight kg	Test Reference range Unit	Ionic calcium 1.12-1.37 mmol/dl	Inorganic Phosphorus 1.13-2.13 mmol/L	Alkaline Phosphatase 1-5.35 ukat/L	25-OH vit D_3 74.88- 249.60 nmol/L	Intact PTH 15-68 ng/L
		Clinical presentation					
21 d	2.4	Admission for hypocalcemic seizures	0.62	2.74	11.2	42.43	6.7
2 mo 10 d	3.6	Hypocalcaemic seizures since birth, on calcitriol- irregular medications -seizures persistent till six weeks after birth	1.12	2.36	9.27	12.23	12
2 mo 27 d	3.7	No seizures in the past 15 d	1.36	2.74	10.3	32	15.4
3 mo 12 d	4.9	Neck holding+, social smile+	1.25	2.26	10.47	NA	17.5
5 mo	6.1	Roll over	1.42	1.65	9.47	144.77	2.01
8 mo 15 d	8.6	Calcitriol gradually tapered and stopped					
38 mo 26 d	12.2	No further seizures. Mental, fine motor and language milestones are normal. Walking at 1.5 y, climbing down stairs with support, riding tricycles	1.24	1.9	4.33	26.21	37.8

PTH, parathyroid hormone; d, days; mo, months; NA, not available; y, years

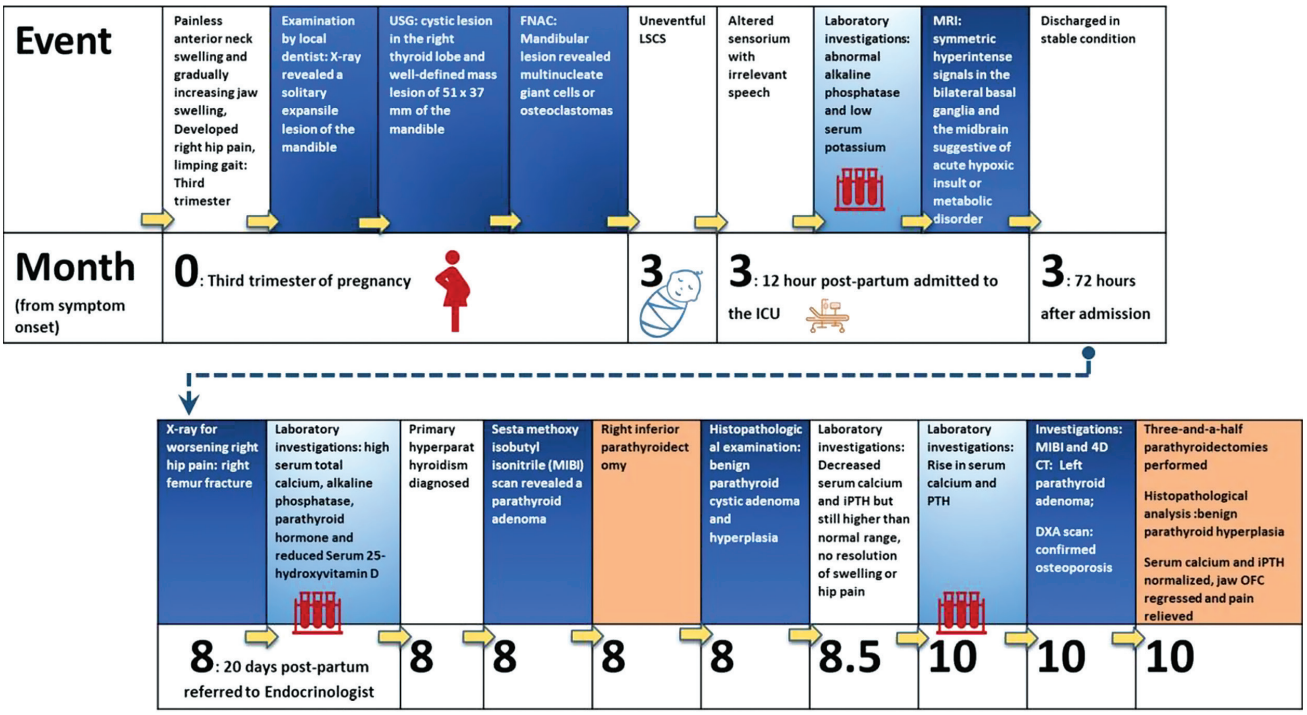

Figure 3. Timeline of events.

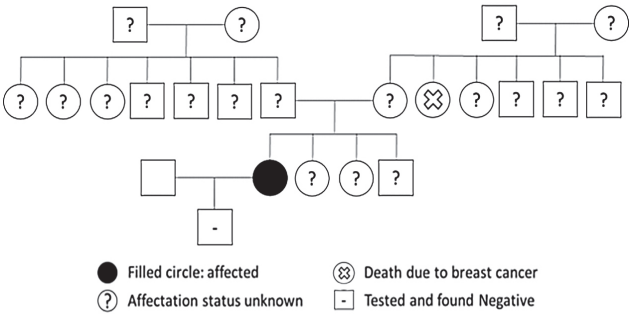

Figure 4. Family tree of the patient with *CDC73* mutation.

Clinical exome sequencing revealed *CDC73*(+) c.687_688dupAG heterogenous pathogenic autosomal dominant mutation.

None of her family members had a history of similar complaints suggestive of PHPT, except for the maternal aunt, who suffered breast carcinoma at 50 years, to which she succumbed (Figure 4). The *CDC73* mutation predisposes the patient to breast cancer and uterine tumors. Given this association, the patient underwent mammography and pelvis USG; however, the reports did not reveal any abnormalities.

The infant was readmitted to the Neonatal Intensive Care Unit (NICU) on day 21 for convulsions. Considering the mother's recent diagnosis of PHPT, the calcium profile was obtained for the neonate, which exhibited low serum ionized calcium of 0.62 mmol/L (1.12-1.37 mmol/L) (Table 3). As per biochemical evaluation, the diagnosis of the neonate was primary hypoparathyroidism leading to hypocalcemic seizures. Calcitriol is used to correct hypocalcaemia in transient or permanent hypoparathyroidism.[11] The infant required calcitriol ((initial dose, 0.40 µg/Kg/d, tapered to 0.25 µg/Kg/d) until 8 months of age, after which it was gradually tapered and stopped.

At 3 years of age, his weight was 13 kg (25th percentile), and his height was 92 cm (25th percentile at mid-parental height). He is currently asymptomatic with slight gross motor delay. Mental, fine motor and language milestones were aligned with normal growth parameters. The genetic testing of the baby for the CDC73 mutation was negative.

DISCUSSION

This case depicts a case of PHPT, a rare disease in pregnancy complicated by an expansile mandibular lesion or OFC of the jaw and femur fracture, possibly due to *CDC73* mutation. Osteitis fibrosa cystica generally occurs due to severe and long-standing hyperparathyroidism.[12] The most common cause of PHPT in pregnancy is predominantly a single adenoma (85% of all cases), followed by primary parathyroid hyperplasia (10% of all cases), multiple adenomas (3%), and parathyroid carcinoma (2%). In our patient, multiple adenomas and hyperplasia were noted.[13] In western regions, where monitoring of calcium and PTH levels is more widespread, PHPT is detected at milder asymptomatic stages like in the form of osteopenia. However, in developing countries like India, PHPT remains undiagnosed until its severe manifestations, like OFC and pathologic fractures, are evident, as found in the current case. Primary hyperparathyroidism also manifests at a younger age than in Western countries, with higher levels of calcium and PTH with larger adenomas, consistent with vitamin D depletion and skeletal PTH resistance.

A previous case report of a 29-year-old female suggested that increased vitamin D and mineral requirements during pregnancy may trigger accelerated bone resorption followed by the emergence of the jaw OFC which may also be the case in our patient.[12]

As in our patient, the skeletal manifestations attributed to reduced BMD were remarkable in the femoral neck. Female gender has been identified as a risk factor for proximal femoral fractures.[14] The diagnosis of PHPT was confirmed post-partum, with investigations primarily prompted by hip pain and subsequent findings of femur fracture on the radiograph. Three-and-a-half gland parathyroidectomies were performed over 3-4 months, and pharmacological treatment resolved biochemical abnormalities and clinical manifestations. This further confirmed the diagnosis of PHPT-associated OFC since HPT-JT ossifying fibromas do not regress with parathyroidectomies. The hungry bone syndrome commonly occurs post-parathyroidectomy in those with concomitant vitamin D deficiency, as found our patient with severe PHPT and moderate vitamin D deficiency.[15]

Neurologic deterioration in the form of irrelevant talk and disorientation was noted in the patient twelve hours post-partum and was attributed to hypercalcemia. Neurologic deterioration due to metabolic encephalopathy secondary to hypercalcemia is known. A sudden increase in serum calcium levels results in neurological symptoms such as decreased concentration, confusion, and rarely stupor or coma. The hypercalcemic crisis is a condition characterized by decompensation of hypercalcemia and predominantly occurs in PHPT as in our patient.[16]

When the infant was admitted with convulsions, the evaluation suggested low serum calcium with (near normal serum phosphorus) with low 25 OH Vitamin D3 with inappropriately low serum intact PTH for 25 OH vitamin D3 and total calcium. Hence, primary hypoparathyroidism was considered as the cause of hypocalcemia rather than vitamin D deficiency. This was the likelier scenario since the mother had undiagnosed PHPT during pregnancy. Maternal hypercalcemia due to PHPT suppresses foetal PTH synthesis and alters the PTH response to post-partum hypocalcaemia in the newborn. This results in congenital neonatal hypoparathyroidism and consequent hypocalcaemia. Severe PTH suppression, therefore, can cause hypocalcaemic convulsions in the neonatal period and may persist for several weeks. In our case, the infant suffered convulsions on day 21, which persisted for 6 weeks.[17] Undiagnosed PHPT in the mother during pregnancy led to an absence of calcium supplementation, resulting in neonatal hypocalcaemic convulsions.[18] Calcitriol was prescribed to the infant (initial dose, 0.40 μg/Kg/d, tapered to 0.25 μg/Kg/d) to maintain serum calcium level in the normal range and avoid hypocalcaemia-related seizures. Convulsion resolved at 6 weeks of age and the infant completely recovered from transient congenital hypoparathyroidism by the age of eight months.

In a large case series published previously, the average calcium level of mothers at diagnosis and in those who experienced pregnancy loss was 2.85 mmol/L while in our patient, the calcium level was 3.33 mmol/L at diagnosis (post-partum) but she had a successful full-term pregnancy.[19] The serum calcium levels were also higher than all the patients in the case series. Moreover, she had no history of previous miscarriages, in contrast to the patients in the case series. The case series emphasized that full-term pregnancy was unlikely with serum calcium >3.0 mmol/L, but the serum calcium was 3.3 mmol/L in our patient.

Guidelines and best practices suggest parathyroidectomy for asymptomatic non-pregnant patients with calcium elevations >1 mg/dL (0.25 mmol/L) above the upper limits of normal (or >11.5 mg/dL; 2.88 mmol/L) and in pregnant females with calcium levels >11.4 mg/dL (2.85 mmol/L).[19] Surgery is a potential treatment option, but it has potential risks during pregnancy. It is reserved for patients in the second trimester, given the higher risk in the first (incomplete organogenesis) and third trimester (higher risk of preterm labour, >50% fetal mortality).[20,21] Therefore, whenever appropriate, conservative medical therapy is utilized based on the risk-benefit ratio.

Genetic testing revealed the presence of the *CDC73* nonsense mutation, a tumor suppressor gene located on chromosome 1q31. *CDC73* c.687_688dupAG mutation results in a frameshift mutation that causes a premature translational stop signal (p.Val230Glu) or nonsense-mediated decay. The p.Val230GlufsTer28 variant has not been reported in the 1000 genomes and the laboratory's internal database and has a minor allele frequency of 0.0008% in the ExAC database. The observed frameshift variant (c.687_688dupAG, p.Val230GlufsTer28) is rare (gnomAD-0.002%). Loss-of-function variants in the *CDC73* gene are a known mechanism of this disease. It may result in an absent or disrupted protein product.[22] Possibly, this female has developed PHPT as the result of a *de novo* pathogenic variant of *CDC73* since none of her family members appear to have similar signs or symptoms or may be asymptomatic. Data on the proportion of individuals with a *de novo* pathogenic variant are currently not known but the variant is not novel.[23] This variant has been previously reported in individuals with hyperparathyroidism-jaw-tumor syndrome and/or isolated hyperparathyroidism.[24] It has also been observed to segregate with disease in related individuals. Based on this evidence and according to the ACMG guidelines (PVS1, PM2, PS4, PP5), this variant has been classified as pathogenic.[25] Due to the predisposition to breast and uterine tumors in patients with *CDC73* mutations, the patient was advised on periodic life-long screening. Genetic testing of the child of the index patient was performed as there is a 50% chance of inheriting the pathogenic variant from the mother, but it was negative.[23]

The family has been counselled regarding the screening; however, it is pending. This is most probably due to

difficulties with out-of-pocket expenses owing to a lack of insurance.

CONCLUSIONS

We present this rare diagnosis of PHPT in a pregnant woman to raise awareness among physicians about this critical differential diagnosis. In patients who exhibit swelling of the neck and jaw, thorough investigations and prompt referral are critical. Simultaneous high levels of PTH and serum calcium should raise a high index of suspicion for PHPT. Parathyroidectomy helps manage the biochemical abnormalities and regresses the jaw's OFC. Parathyroidectomy also helps in attenuating the declining BMD. Children born to mothers with PHPT should be evaluated for neonatal hypoparathyroidism and supplemented appropriately to reduce the risk of hypocalcaemic manifestations that can be life-threatening. Genetic testing in young PHPT would help strategize management for the proband as well as the family early in the disease.

Learning Points

- In the case of altered sensorium in the peripartum period, serum calcium should be determined for early diagnosis.
- Hyperparathyroidism is a rare diagnosis, and a high index of suspicion is necessary when a patient presents with a jaw lesion. Endocrine evaluation should be performed in addition to a dental examination.
- Long-term follow-up of the child is required to determine whether he completely recovered and did not develop permanent hypoparathyroidism.

Patient Perspective
The female patient feels satisfied with the regression of her jaw and neck swelling, which significantly altered her appearance. She is completely relieved of her bone pain. The infant is currently asymptomatic and displays normal growth. The patient feels that the correct diagnosis helped obtain appropriate treatment.

Ethical Consideration
Patient consent was obtained before submission of the manuscript.

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

CRediT Author Statement
PP: Conceptualization, Methodology, Investigation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **AN:** Investigation, Resources; **YC:** Formal analysis, Investigation, Data Curation, Writing – original draft preparation, Project administration; **PV:** Investigation, Writing – review and editing, Supervision; **NB:** Investigation, Resources, Supervision.

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Author Disclosure
The authors declared no conflict of interest.

Data Availability Statement
No datasets were generated or analyzed for this study.

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CASE REPORT

Journal of the ASEAN Federation of Endocrine Societies

Aggressive Synchronous Papillary and Likely Follicular Thyroid Carcinomas in a Patient with Graves' Disease

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Abstract

We report a case of an uncommonly aggressive presentation of the rare entity of synchronous papillary (PTC) and follicular thyroid carcinomas (FTC) in a 67-year-old female initially presenting with thyrotoxicosis from Graves' disease. She was found to have two thyroid nodules with extensive intra-cardiac tumour thrombus, symptomatic left pelvis bony metastasis with pathological fracture, pulmonary metastases and mediastinal lymph node metastases. Further investigations suggested a diagnosis of synchronous papillary and metastatic follicular thyroid cancer. Treatment with radical surgery followed by adjuvant therapeutic radioiodine ablation was proposed, but the patient declined all forms of cancer-specific therapy and was elected solely for a palliative approach to treatment. We discuss the diagnostic considerations in arriving at the diagnosis of synchronous thyroid malignancy – in this case the clear features of PTC and the strong probability of FTC due to invasiveness and metastatic follicular lesions. This case underscores potential limitations of the ACR TI-RADS system, notably with certain ultrasonographic features suggesting malignancy that might not be adequately captured. Notably, the aggressive presentation of DTC in this case may be contributed by the concurrent presence of Graves' Disease, suggesting heightened vigilance when assessing potential thyroid malignancies in such patients.

Key words: papillary thyroid carcinoma, follicular thyroid carcinoma, tumour thrombus, Graves' disease

INTRODUCTION

Thyroid cancer is the most common endocrine malignancy, with differentiated thyroid carcinoma (DTC), comprising papillary, follicular and Hurthle cell subtypes, accounting for 90% of cases.[1] However, the simultaneous occurrence of papillary and follicular thyroid carcinomas is extremely rare. This case report presents an unusual and aggressive case of synchronous papillary and likely follicular thyroid carcinomas with Graves' disease and discusses the diagnostic challenges, management decisions and clinical implications.

CASE

A 67-year-old female with no significant past medical history presented to the emergency department with symptoms of bilateral lower limb swelling for a week, unintentional weight loss of 6 kg, heat intolerance, palpitations, anxiety and intermittent mild facial swelling. She reported a secondary concern of left buttock pain radiating to the left thigh for 2 weeks, with no preceding trauma. She denied any smoking history or family history of thyroid disease or malignancy.

Upon examination, she was tachycardic and hypertensive but afebrile and not hypoxic. Bilateral pitting edema to the mid-shin was noted and she was mildly tremulous. However, she had no proximal weakness or thyroid eye disease. She had an asymmetrical goiter, larger on the right side, with a palpable, non-tender, firm nodule measuring approximately 2 x 2 cm. A markedly distended right external jugular vein was also noted. There was no cervical lymphadenopathy discernible clinically. Lastly, she did not have any pelvic tenderness nor pain on axial loading of her left hip and neurological examination of both lower limbs was unremarkable with a negative straight-leg raise test.

Diagnostic assessment

Initial investigations revealed primary hyperthyroidism with free thyroxine (FT4) level of 22.7 pmol/L [reference range 8.8-14.4 pmol/L] and completely suppressed thyroid stimulating hormone (TSH) levels of less than 0.010 mU/L [reference range 0.65-3.70 mU/L]. She had a positive TSH receptor antibody level of 4.57 IU/L [reference range <1.76 IU/L] and was therefore diagnosed with Graves' disease. Liver and renal function and full blood count were normal.

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N-terminal pro-brain natriuretic peptide was elevated, but troponin levels were not raised on serial measurement. Chest x-ray showed cardiomegaly but no pulmonary congestion or lung lesions. Lumbar spine, pelvis and left hip radiographs were unremarkable. An electrocardiogram showed sinus tachycardia with the "S1Q3T3" triad, but no right axis deviation.

A transthoracic echocardiogram showed normal left ventricular structure and function without significant valvulopathy. However, an echodense mass measuring 4.4 x 2.4 cm was reported in the right atrium, with apparent extension into the inferior vena cava (Figure 1). Urgent computed tomography pulmonary angiography did not show any evidence of pulmonary embolism. The hypodense right atrial mass was further characterized (5.3 x 3.2 x 9.0 cm in dimension) and was demonstrated to extend up the superior vena cava (SVC) (Figure 2A). Asymmetric enlargement of the right thyroid lobe was observed, with associated nodularity and dystrophic calcification (Figure 2B). Lastly, multiple pulmonary nodules were noted in the left lower lobe (1.2 cm), left upper lobe (0.6 cm) and right lower lobe (0.4 cm).

Ultrasonography of the thyroid gland (US Thyroid) showed 2 thyroid nodules. The first was a heterogenous hyperechoic solid isthmic nodule measuring 1.7 x 1.6 x 1.4 cm (wider than tall) with ill-defined margins, classified as TIRADS-3 (Figure 3A). The second was a solid iso-to-hypoechoic solid right lower pole nodule measuring 2.0 x 2.0 x 1.8 cm (wider than tall) with a disrupted eggshell calcification pattern, classified as TIRADS-4 (Figure 3B). This corresponded to the nodule observed on CT. A right internal jugular vein (IJV) thrombus measuring 3.4cm with internal vascularity suggestive of tumour thrombus was reported (Figure 3C). Lastly, an abnormal lymph node was noted at the right submental/submandibular region (Level IA/B) measuring 3.5 x 1.1 x 1.7 cm and demonstrating internal vascularity (Figure 3D). Increased vascularity of the thyroid parenchyma was noted, in keeping with Graves' disease.

Fine needle aspiration of both thyroid nodules and the right submental lesion was performed. Cytology of the isthmic nodule showed features consistent with papillary thyroid carcinoma (PTC) (Bethesda VI) with classic microscopic features of pale nuclei with intranuclear pseudoinclusions and nuclear grooves (Figure 4A and 4B). Cytology of the right lower pole nodule showed follicular cells predominantly arranged in microfollicles and trabeculae (Figure 4C and 4D) characteristic of a follicular neoplasm (Bethesda IV). Cytology of the right submental lesion showed metastatic epithelial cells arranged in trabeculae and follicles, without classic nuclear features of PTC, in keeping with metastasis from the follicular neoplasm at the right lower pole (Figure 4E and 4F).

Staging was completed with computed tomography of the neck, thorax, abdomen and pelvis. The right IJV thrombus was further delineated to extend superiorly up to the right common facial, facial, anterior jugular and superior thyroid veins and inferiorly down to the right brachiocephalic vein and SVC. This thrombus was noted to be inseparable from the superior margin of the right thyroid gland (Figure 5A). The right submental lymph node previously reported on US Thyroid was suggested to instead represent the thrombosed right anterior jugular vein (Figure 5B). Mediastinal lymphadenopathy was also noted. A soft tissue mass measuring 4.9 x 4.7 cm centred on the left ilium with bony destruction and pathological fracture was seen (Figure 5C). This was the most likely cause of her reported left buttock pain. Lastly, a cardiac MRI was performed. The right atrial mass showed internal enhancement with gadolinium contrast, suggesting internal vascularity and hence tumour (as opposed to bland) thrombus.

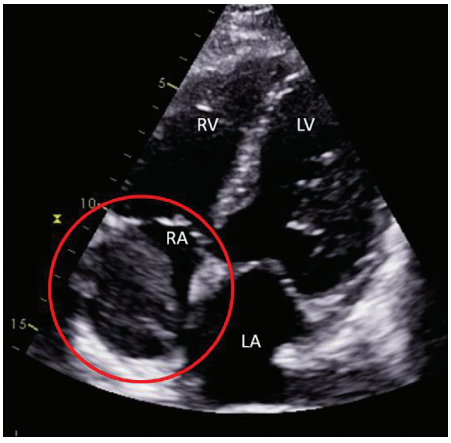

Figure 1. Apical 4-chamber view of transthoracic echocardiogram. The echodense mass is highlighted with the red circle.

RV = Right Ventricle, LV = Left Ventricle, RA = Right Atrium, LA = Left Atrium.

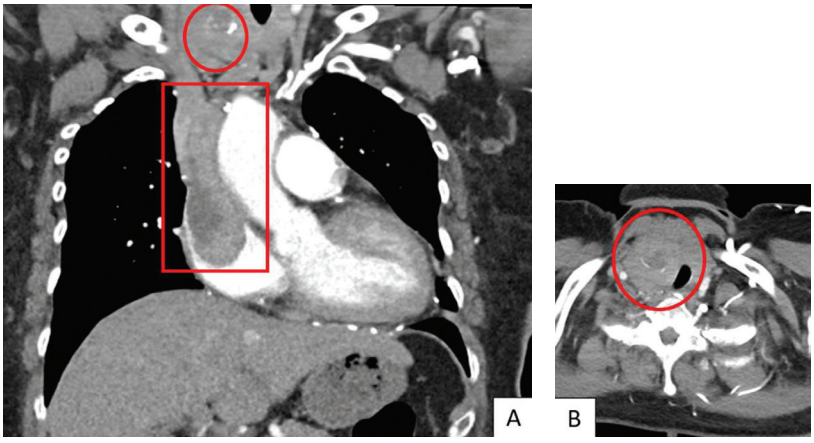

Figure 2. (A) Coronal view showing extensive RA thrombus with SVC extension *(red box)* and nodular calcification in thyroid *(red circle)*. **(B)** Axial view showing asymmetric thyroid enlargement with calcified right thyroid nodule *(red circle)*.

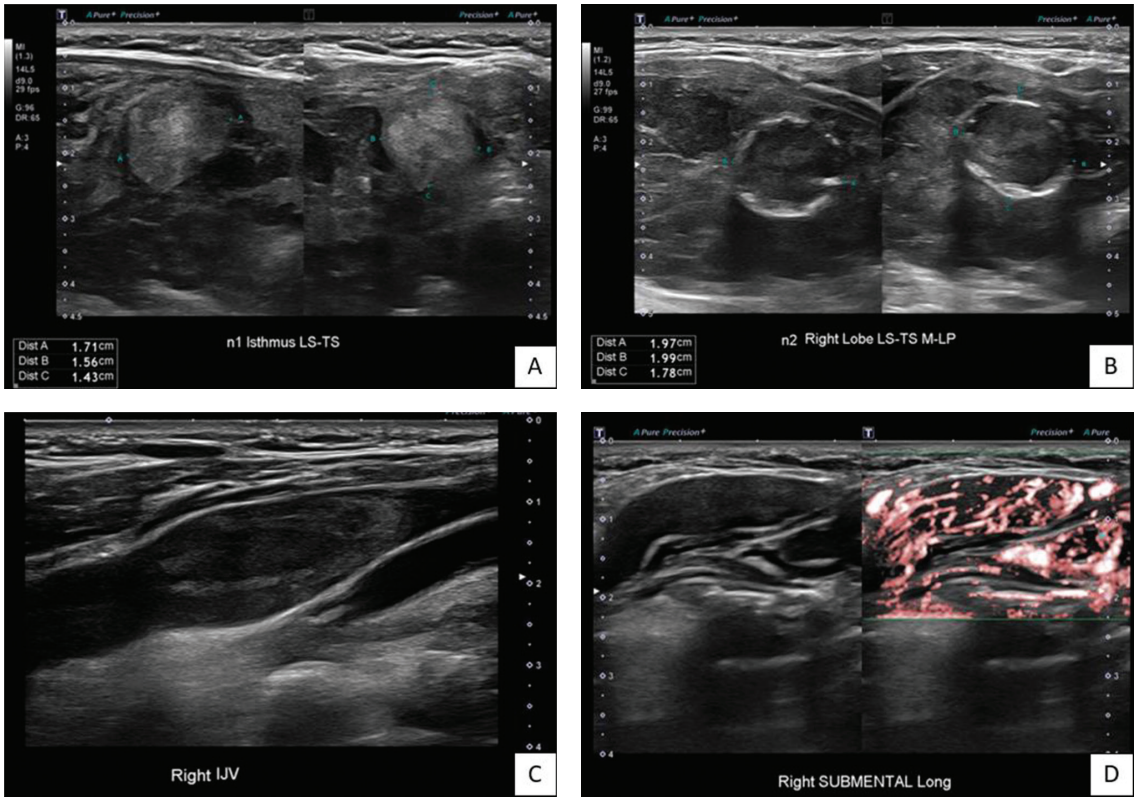

Figure 3. (A) Isthmic nodule **(B)** Right thyroid lower pole nodule **(C)** Right internal jugular vein thrombus **(D)** Right submental lesion with internal vascularity.

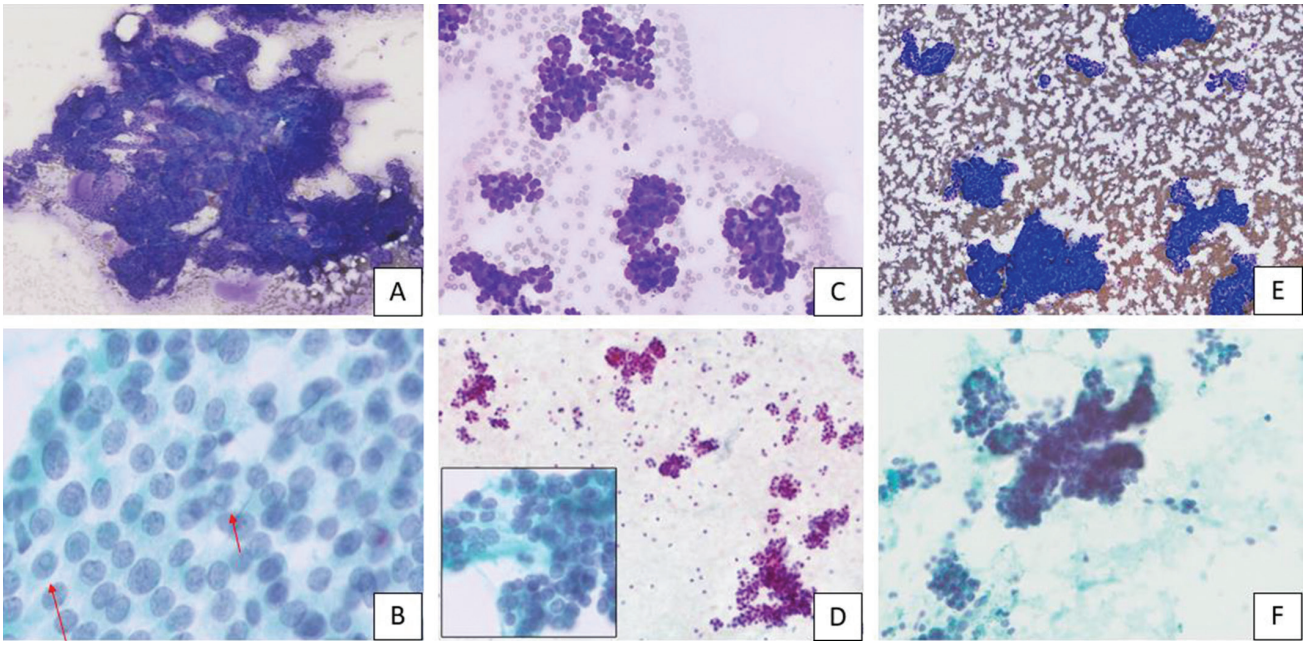

Figure 4. (A) Diff Quik; 5x magnification – Papillary clusters of thyroid follicular cells. **(B)** Papanicolaou; 40x magnification – Follicular cells from papillary thyroid carcinoma showing pale ovoid nuclei with intranuclear pseudoinclusions *(arrows)* and nuclear grooves. **(C)** Diff Quik; 10x magnification – Thyroid follicular cells arranged in microfollicles and trabecular groups. **(D)** Papanicolaou; 5x magnification – Predominance of microfollicles. Follicular cells from "nodule 2" have round nuclei with evenly dispersed, granular chromatin (inset, 40x magnification). **(E)** Diff Quik; 5x magnification – Metastatic epithelial cells arranged in trabeculae and crowded clusters. **(F)** Papanicolaou; 10x magnification – Metastatic epithelial cells arranged in trabeculae and microfollicles.

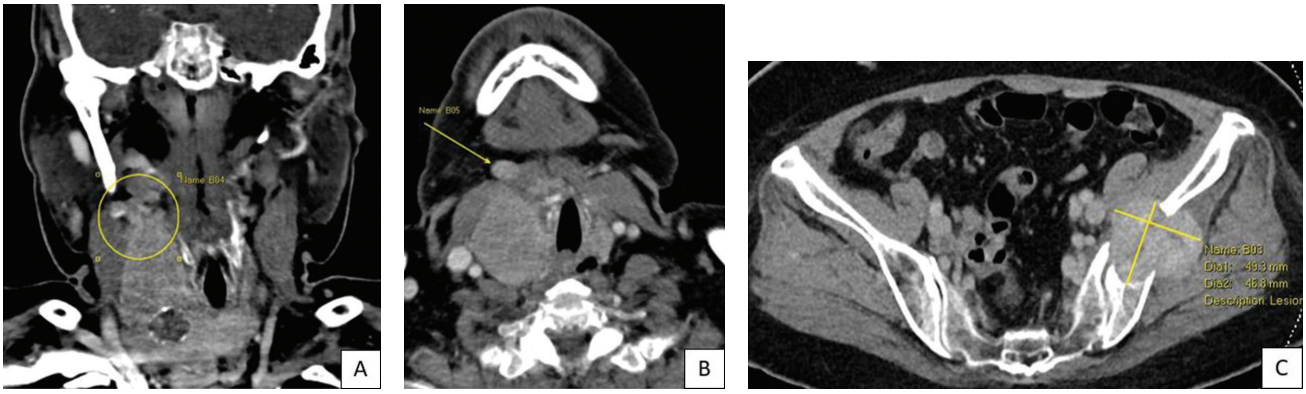

Figure 5. CT Neck **(A)** Coronal view showing extensive tumour thrombus inseparable from superior border of right thyroid lobe *(yellow circle)*. **(B)** Axial view showing the abnormal right submental structure, either thombosed anterior jugular vein or submental lymph node. **(C)** Destructive bony metastasis in left ilium.

The final diagnosis for this patient was a synchronous papillary and likely follicular thyroid cancer (FTC), complicated by extensive tumour thrombus from the right common facial vein to the right atrium, a symptomatic left pelvic bony metastasis with pathological fracture and likely pulmonary, mediastinal and possibly right submental lymph node metastases. This was on a background of primary hyperthyroidism secondary to Graves' disease.

Treatment and outcome

The patient was started on oral carbimazole 20 mg once daily at the time of admission. The beta-adrenergic blockade was withheld in view of the initial suspicion of acute decompensated heart failure. Her FT4 levels normalized by the time of her discharge from the hospital.

A multidisciplinary tumour board recommended total thyroidectomy with lateral neck dissection, followed by median sternotomy, cardiopulmonary bypass, venotomy and thrombectomy – an approach previously reported in a similar case.[2] After consideration of the risks of surgery against the likelihood of a poor outcome without surgery and possible sudden cardiac death in the event of tumour embolization, the patient elected against proceeding with the recommended surgical resection and also declined further discussion of chemotherapy or molecularly-targeted therapy.

She remains on ongoing follow-up for the last 18 months since the time of her diagnosis. TRAb levels normalized rapidly and carbimazole was discontinued 8 months after her initial presentation due to persistent hypothyroidism despite rapid progressive reductions in carbimazole doses. A repeat US Thyroid was arranged 6 months after her diagnosis, which showed further tumour progression in the thyroid gland, possibly the cause of her progressive hypothyroidism. A repeat transthoracic echocardiogram also showed further progression of the right atrial mass with right ventricular inflow tract obstruction. Despite extensive counselling on the possibility of sudden cardiorespiratory collapse, she remained opposed to treatment. Overall management remains expectant, and she is mentally prepared for the likelihood of a poor eventual outcome.

DISCUSSION

Synchronous PTC and FTC are exceedingly rare, with fewer than 10 cases reported in the extant literature.[3,4] Whilst also rare, synchronous papillary and medullary thyroid carcinomas (MTC) are better reported.[5] This is thought to be due to the differences in the molecular pathogenesis of PTC and FTC4. PTC is more commonly associated with BRAF mutations (40-45%) and RET/PTC (10-20%) fusions and thus shares homologous mutations with MTC (germline RET/PTC mutations in patients with MEN2 and 40-45% of patients with sporadic MTC). FTC on the other hand is more associated with mutations of RAS (40-50%) and PAX8/PPARγ fusions (30-35%).[6]

The diagnosis of PTC in this case is clear, given the presence of distinctive nuclear features on cytology as described. However, the conclusive diagnosis of FTC (as opposed to follicular adenoma) requires the demonstration of invasiveness. Hence, surgical resection and subsequent microscopic examination are necessary to make the diagnosis. Despite the lack of conclusive histopathology, there are a few supporting features that make this a strong possibility. Firstly, the abnormal right thyroid lobe is inseparable from the tumour thrombus in the right IJV, suggesting invasiveness. Secondly, the right submental lesion demonstrates clear follicular cytopathology without any nuclear features of PTC. As this is a metastatic lesion, by logical extension, a metastatic follicular lesion would necessarily be a FTC. Lastly, the presence of bony metastasis at the time of diagnosis is a clinical feature more closely associated with FTC.[7] The differential diagnosis of a follicular-variant PTC was considered less likely given the lack of nuclear features of PTC. There were also no features of poorly differentiated thyroid carcinoma.[8]

This case also highlights potential limitations of the American College of Radiology Thyroid Imaging Reporting and Data System (ACR TI-RADS).[9] Using the ACR TI-RADS

system, the isthmic nodule was classified as TIRADS-3 and the right thyroid nodule was classified as TIRADS-4, which would confer a cancer risk of <5% and 5-20% respectively[9] in the absence of other suspicious ultrasonographic features. In particular, the appearance of the right thyroid nodule should be considered. Peripheral or "eggshell" calcifications have classically been considered a feature suggestive of benignity.[10] However, more recent evidence suggests that in nodules without any other internal calcifications, disruption of the peripheral calcification and a peripheral halo are much more predictive of malignancy than other features captured by ACR TIRADS such as hypoechogenicity, lobulated margins or a taller-than-wide shape.[11] Both these features were observed in this case. Additionally, the isthmic nodule would have been recommended for surveillance alone by size (<2.5 cm) and TIRADS-3 classification.

Secondly, this was an unusually aggressive presentation of DTC. It is rare to observe tumour thrombi at the time of DTC diagnosis, with a large observational series reporting a prevalence of less than 1%.[12] TSH is a key stimulator of DTC growth, which explains the rationale for TSH suppression in patients at risk of recurrent disease after initial treatment. TSH receptor antibodies are homologous to TSH and can activate cellular TSH receptors in the same way. It has therefore been suggested that the presence of concomitant Graves' disease might be associated with increased aggressiveness of thyroid cancer at the time of presentation, with higher rates of multifocality, local invasion and metastases.[13,14] It may be prudent to be more cautious when evaluating and treating possible thyroid malignancies in patients with Graves' disease.

CONCLUSION

This case presents an exceptionally aggressive manifestation of rare synchronous PTC and likely FTC in a patient with Graves' disease, illustrating some diagnostic challenges and the potential influence of Graves' disease on thyroid malignancy severity.

Ethical Consideration
Patient consent was obtained before submission of the manuscript.

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Journal of the ASEAN Federation of Endocrine Societies

LETTER TO THE EDITOR

In response to the article, *'Hypothalamic-Pituitary-Adrenal Axis Activity in SARS-CoV-2 Infected Noncritically Ill Hospitalized Patients,'* by Banu H, et al., published in JAFES Vol. 38 No. 2.

Before Low Serum Cortisol Can Be Attributed to SARS-CoV-2 Infection, Alternative Causes Must Be Ruled Out

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Key words: SARS-CoV-2 infection, cortisol, ACTH, adrenal insufficiency, COVID-19

We read with interest Banu et al.,'s article about a cross-sectional study of adrenal function using blood levels of cortisol and adrenocorticotropic hormone (ACTH) in 91 patients with acute SARS-CoV-2 infection. It was found that 27% of patients with severe COVID-19, 26% with mild COVID-19 and 4% with moderate COVID-19 had adrenal insufficiency. Decreased cortisol reserve was found in 6.6% of all patients. It was concluded that the adrenocortical response was impaired in a significant number of non-critically ill COVID-19 patients, with the percentage being highest in patients with severe COVID-19 disease.[1] The study is impressive, but some points should be discussed.

The major limitation of the study is that imaging of the pituitary gland and hypothalamus was not performed. To rule out pituitary adenoma, hypophysitis, pituitary apoplexy, pituitary abscess, sellar tuberculoma, or hypothalamic stroke or bleeding, magnetic resonance imaging (MRI) of the pituitary gland and the hypothalamus with contrast agent must be performed. Like any other infection, SARS-CoV-2 infection can trigger the appearance of symptoms of a pituitary pathology.[2]

A second limitation is that imaging of the suprarenal glands was not performed. To assess whether there was adrenal atrophy, adenoma, ischemia, bleeding, or carcinoma, adrenal imaging is mandatory.[3]

A third limitation is that current medication was not reported. Several medications are known to affect serum cortisol levels, making it imperative to know all medications regularly taken by the included patients. Ketoconazole, isilodrostat, vitamin D and omega-3 fatty acids in particular are known to be able to reduce cortisol levels in the blood.[4] A fourth limitation is that no ACTH stimulation test was performed to determine whether primary or secondary suprarenal insufficiency was present. Determining cortisol levels alone without ACTH stimulation testing is not reliable with regard to cortical adrenal function.

A fifth limitation is that serum dehydroepiandrosterone (DHEA) levels were not measured. Since DHEA is the antagonist of cortisol and decreases with age, it is conceivable that high levels of DHEA promoted the reduction of serum cortisol.[5]

Since low thyroxine levels may be associated with low cortisol levels,[6] it would also be desirable to know whether thyroid function was normal or abnormal in the included patients.

In summary, the excellent study has limitations that should be addressed before drawing conclusions. Clarifying the weaknesses would strengthen the conclusions and could improve the study. Serum cortisol levels may be low not only due to SARS-CoV-2 infection but also due to several other causes. Before adrenal insufficiency can be attributed solely to inadequate cortical cortisol production, the entire hypothalamic-pituitary-adrenal axis must be thoroughly examined.

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LETTER TO THE EDITOR

In response to the article, *'A Cross-sectional Study to Assess Beta-Cell Function in Individuals with Recently Diagnosed Young-Onset Type 2 Diabetes Mellitus and Its' Complications,'* by Nagaratnam S, et al., published in JAFES Vol. 38 No. 2.

Other Diagnostic Tests for Young-Onset Type 2 Diabetes Mellitus

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We have read with interest the article entitled "A Cross-sectional Study to Evaluate Beta Cell Function in Individuals with Newly Diagnosed Young-Onset Type 2 Diabetes Mellitus and its Complications,"[1] elevated C-peptide levels are indicative of both insulin resistance and beta cell malfunction. These factors are directly linked to the development of complications associated with young-onset Type 2 Diabetes Mellitus (YO-T2DM), including obesity, hypertension, and diabetic kidney disease. The present letter aims to expand the current diagnostic testing options for early detection of YO-T2DM.

The article of Virostko[2] mentions that magnetic resonance (MR) has demonstrated its usefulness as a technique for image analysis that can accurately characterize the heterogeneity of the pancreas among patients with Type 1 and 2 Diabetes Mellitus, where excess pancreatic fat affects its complex structure, ranging from the pancreatic duct network to the islets of the acinar cells. This would be an indicator of decreased beta cell function, which correlates with T2DM. It should be emphasized that this test is more expensive, but with greater specificity for early diagnosis and reduces the risk of future complications.

Genetic testing is also available for the early diagnosis of YO-T2DM. As observed in the article by Wiebe et al.,[3] the sequence of genes such as TCF7L2, KNCJ11 and PPARG1 are predictors for the development of YO-T2DM. The first gene (TCF7L2) encodes insulin-related proteins, which showed great association with the development of YO-T2DM. The PPARG gene, which is related to adipogenesis and insulin resistance, showed a direct association with YO-T2DM. The KNCJ11 gene encodes beta-cell potassium channel receptors associated with sulfonylurea SUR1 receptors, associated with the development of YO-T2DM. Thus, these genetic sequences could provide specific coding that evidences the risk of developing YO-T2DM because they are directly related to the regulation of glucose metabolism, insulin resistance and beta cell function. The disadvantage, however, is that this type of diagnosis is more expensive, but provides greater specificity.

Epidemiological studies reveal a concerning rise in YO-T2DM prevalence in Southeast Asia, increasing from 0.19% (2007) to 0.43% (2018).[4] Combining multiple assessment methods could provide a more complete understanding of beta cell functionality in this population. Thus, the implementation of updated diagnostic methods, such as MRI and genetic testing, represents a crucial advance in the diagnosis of patients with YO-T2DM. These tools facilitate a more accurate diagnosis to facilitate early intervention, which helps circumvent serious complications in the future and optimize the quality of life of these individuals.

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As part of good publication practices, all editors of the JAFES (Editor-in-Chief, Vice Editor-in-Chief, Associate Editors, Editorial Board and Advisers) are required to disclose on an annual basis, any potential conflict of interest, including current or recent financial relationships with any commercial entity whose products or services may be contained in the journal content. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. This policy follows the recommendations of the International Committee of Medical Journal Editors (ICMJE) and the Committee on Publication Ethics (COPE).

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Prof. Deerochanawong has nothing to disclose.

NGUYEN THY KHUE | Associate Editor
Prof. Khue receives consulting fees from Merck. She receives honoraria for lectures from Servier, Boehringer Ingelheim and Novo Nordisk. She receives fees as the Advisory Board of Kowa. She is a member of the Vietnamese Association of Diabetes and Endocrinology (VADE), Ho Chi Minh City Association of Diabetes and Endocrinology (HADE) and Ho Chi Minh City Medical Association.

MARY ANN R. ABACAN | Editorial Board Member
Prof. Abacan receives grants as co-investigator of the Registry of Lysosomal Storage Disorders from Sanofi Genzyme (2014 to present) and as co-investigator of the SHP-ELA-401 - A Long-Term, Open-Label, Multicenter, Phase IV Study to Assess Longitudinal Changes on Height and Weight in Patients with MPS II who are receiving Elaprase and started Treatment with Elaprase at <6 Years of Age - Shire (2015-present). She is a Consultant Medical Specialist at the Institute of Human Genetics at the University of the Philippines-National Institutes of Health. She received honoraria as a lecturer of the Training Workshop for Dietitians (Overview of Urea Cycle Disorders). She is a co-chair of the National Transplant Ethics Committee of the Department of Health and the Chair of the Metabolic Experts Panel at the Newborn Screening Reference Center of the National Institutes of Health. She is a stockholder of Clinical Genetics and Genomics Counselling Care Services, Inc. She is the Chair of the Congenital and Developmental Disorders Task Force, Philippine Periodic Health Examination II - Institute of Clinical Epidemiology and DOH joint project (2021), Technical Facilitator of the Pediatric Immunization Task Force, Philippine Periodic Health Examination III - Institute of Clinical Epidemiology and DOH joint project (2023) and a Technical Facilitator of the Dengue Task Force, Philippine Periodic Health Examination III - Institute of Clinical Epidemiology and DOH joint project (2023).

LORNA R. ABAD | Editorial Board Member
Prof. Abad has nothing to disclose.

MARISSA M. ALEJANDRIA | Editorial Board Member
Prof. Alejandria has nothing to disclose.

PIA D. BAGAMASBAD | Editorial Board Member
Prof. Bagamasbad received grant funding from the Department of Science and Technology-Philippine Council for Health Research Development (DOST-PCHRD) to conduct research on prostate cancer and SARS-CoV-2 and the DOST-National Academy of Science and Technology (NAST) to conduct research on prostate cancer. She received honoraria as a plenary speaker of the Philippine College of Endocrinology, Diabetes and Metabolism (PCEDM) and the Philippine Society of Medical Oncology (PSMO). She is a member of the Endocrine (ENDO) Society (no financial involvement).

SARAT SUNTHORNYOTHIN | Editorial Board Member
Prof. Sarat has nothing to disclose.

NATTAPOL SATHAVARODOM | Editorial Board Member
Prof. Nattapol has nothing to disclose.

CHIAW LING CHNG | Editorial Board Member
Dr. Chng has nothing to disclose.

TINT SWE LATT | Editorial Board Member
Prof. Latt has nothing to disclose.

KHOO CHIN MENG | Editorial Board Member
Dr. Meng has nothing to disclose.

NURAIN MOHD NOOR | Editorial Board Member
Dr. Noor is the current President of the Malaysian Endocrine and Metabolic Society (MEMS).

NATHANIEL S. ORILLAZA JR. | Editorial Board Member
Dr. Orillaza receives research grants from the University of the Philippines and the Department of Science and Technology-Philippine Council for Health Research Development (DOST-PCHRD). He receives honoraria for lectures from Viatris, Mundipharma and Menarini. He receives faculty development grants from the University of the Philippines and financial support from Viatris to attend a pain management conference. He applied for patents for medical devices at the University of the Philippines. He is a Research Officer of the AO Trauma Philippines, an evidence reviewer of the CPG Development team for the Osteoporosis Society of the Philippines Foundation Inc. (OSPFI) and a Steering Committee member of the CPG Development team for The Philippine Guidelines on Periodic Health Examination for Screening of Musculoskeletal Disorders (PHEX MSK). He is a stockholder of the Tagaytay Medical Center.

PAUL MATTHEW D. PASCO| Editorial Board Member
Dr. Pasco has nothing to disclose.

CATHERINE LYNN T. SILAO| Editorial Board Member
Dr. Silao receives research grants from the Newborn Screening Reference Center and the National Institutes of Health. She receives fees for lectures from the Nestle Nutrition Institute. She received grants from the Asia Pacific Society of Human Genetics. She has pending patents for Methods and Means for Prognosticating the Occurrence of Pulmonary Complications in Leptospirosis and Early Diagnosis and Prognosis of Complicated Leptospirosis using Molecular Markers. She is the Secretary and Board of Directors of the Asia Pacific Society of Human Genetics. She is also a member of the Human Genome Education Committee (no financial involvement). She is a stockholder of the Clinical Genetics and Genomic Counseling Care Services (CGGCCS) Inc.

ROGELIO V. TANGCO | Editorial Board Member
Dr. Tangco has nothing to disclose.

NGUYEN VAN TUAN | Editorial Board Member
Prof. Van Tuan received financial support from the Australian National Health and Medical Research Council for his research. He also received a grant from Amgen to conduct research in osteoporosis. He is a member of Healthy Bone Australia (no financial involvement).

MYO WIN | Editorial Board Member
Prof. Win has nothing to disclose.

MARITA V.T. REYES | Ethics Adviser
Dr. Reyes received a research grant to the Women's Health Care Foundation from the Philippine Council for Health Research and Development. She receives honoraria as a Technical Reviewer of research proposals and as a trainer on research ethics from the Department of Science and Technology-Philippine Council for Health Research Development (DOST-PCHRD). She was also the President and Chair of the Board of Trustees of the Women's Health Care Foundation Incorporated (no financial involvement).

AMADO O. TANDOC III | Editorial Coordinator
Dr. Tandoc receives fees as a Consultant Editor of the Scientific Proceedings of the Lung Center of the Philippines, Philippine Journal of Orthopaedics and the Philippine Journal of Allergy, Asthma and Immunology. He is also the Editor-in-Chief of the Philippine Journal of Pathology. He is a speaker and Treasurer of the Philippine Association of Medical Journal Editors (PAMJE).

Journal of the
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Endocrine Societies

Instructions to Authors

The **Journal of the ASEAN Federation of Endocrine Societies (JAFES)** is an open-access, peer-reviewed, English language, medical and health science journal that is published two times a year by the ASEAN Federation of Endocrine Societies (AFES). **Authors may include members and non-members of the AFES.**

Manuscripts, correspondences and other editorial matters should be sent via electronic mail to JAFES@asia.com or JAFES.editor@gmail.com.

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JAFES welcomes manuscripts on all aspects of endocrinology and metabolism in the form of original articles, review articles, case reports, feature articles (clinical practice guidelines, clinical case seminars, book reviews, et cetera), editorials, letters to the Editor, brief communications, images in endocrinology and special announcements. See Inset Box for descriptions and specific requirements per article type.

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A cover letter must accompany each manuscript which should cite the title of the manuscript, the list of authors (complete names and affiliations and their specific role/s in writing the manuscript), with one (1) author clearly designated as correspondent, providing his/her complete postal/mailing address, telephone number, e-mail and fax number. The **JAFES cover letter template** must be used.

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To improve and standardize reporting of findings depending on the study type, authors should ensure compliance with and submit the appropriate accomplished EQUATOR (Enhancing the QUAlity and Transparency of Research) Network Guidelines. These guidelines are freely available at: http://equator-network.org.

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2. CARE (2013) Checklist for Reporting Case Reports
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In order to ensure scientific objectivity and independence, the JAFES requires all authors to make a full disclosure of areas of potential conflict of interest. Such disclosure will indicate whether the person and/or his/her immediate family has any financial relationship with pharmaceutical companies, medical equipment manufacturers, biomedical device manufacturers, or any companies with significant involvement in the field of health care.

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For Case Reports, Images in Endocrinology and Clinical Case Seminars, authors are required to submit scanned soft copy of signed informed consent for publication from the involved subject/s ("Patient Consent Form"). In case the involved subject/s can no longer be contacted (i.e., retrospective studies, no contact information, et cetera) to obtain consent, the author must seek ethical clearance from the institutional board to publish the information about the subject/s.

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For purposes of transparency and scientific integrity, JAFES requires that authors provide a statement on the availability of data described in the manuscript submission. Depending on the circumstances, one of the following data availability and sharing statements may be selected:

- Datasets generated and analyzed are included in the published article.
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- Dryad [https://datadryad.org/]
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Authors are required to disclose their use of generative artificial intelligence (AI) and AI-assisted technologies for all submissions to JAFES. This shall be described in the cover letter AND the submitted work in the appropriate section as to how it was used.

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1. The manuscript should be encoded using Microsoft Word, double-spaced throughout with 1¼ cm (½ inch) paragraph indentation, with 3-cm margins (1¼ inch) all around on A4 size paper. The preferred font style and size is Times New Roman 12.
2. The manuscript should be arranged in sequence as follows: (1) Title Page, (2) Abstract, (3) Text, (4) References, (5) Tables, and (6) Figures & Illustrations.
3. References should pertain directly to the work being reported.
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5. All manuscripts not complying with the above shall be promptly returned for correction and resubmission.

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1. The title should be as concise as possible.
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   2.1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
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3. The highest educational attainment or title of the authors should be included as an attachment whenever appropriate.
4. Name and location of no more than one (1) institutional affiliation per author may be included.
5. If the paper has been presented in a scientific forum or convention, a note should be provided indicating the name, location and date of its presentation.

Abstract
For original articles, the abstract should contain no more than 200 words with a structured format consisting of the objective/s, methodology, results and conclusion. For feature articles, case reports, interhospital grand rounds, and brief communications, the abstract should be from 50 to 75 words and need not be structured.

Keywords
At least 3 keywords but no more than 6, preferably using terms from the Medical Subject Headings (MeSH) list of Index Medicus, should be listed horizontally under the abstract for cross-indexing of the article.

Text
1. Generally, the text should be organized consecutively as follows: Introduction, Methodology, Results and Discussion, and Conclusion (IMRAD format).
2. All references, tables, figures and illustrations should be cited in the text, in numerical order.
3. All abbreviations should be spelled out once (the first time they are mentioned in the text) followed by the abbreviation enclosed in parentheses. The same abbreviation may then be used subsequently instead of the long names.
4. All measurements and weights should preferably be in System International (SI) units.
5. If appropriate, information should be provided on institutional review board/ethics committee approval.
6. Acknowledgments to individuals/groups of persons, or institution/s should be included at the end of the text just before the references. Grants and subsidies from government or private institutions should also be acknowledged.

References
1. References in the text should be identified by Arabic Numerals in superscript on the same line as the preceding sentence.
2. References should be typed double-spaced on a separate sheet. They should be numbered consecutively in the order by which they are mentioned in the text. They should not be alphabetized.
3. All references should provide inclusive page numbers.
4. Journal abbreviations should conform to those used in PubMed. Include PMID, PMCID and DOI of the references.
5. A maximum of six authors per article can be cited; beyond that, name the first three and add "et al."
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Journal Article
Padua FR, Paspe MG. Antinuclear antibody in the rheumatic and non-rheumatic diseases among Filipinos. Acta Med Philipp. 1990; 26(2):81-5.

One to Six Authors (Commentary, Online)
Krause RM. The origin of plagues: Old and new. Science. 1992;257:1073-8. PMID: 1509258 DOI: 10.1126/science.257.5073.1073.

Barry JM. The site of origin of the 1918 influenza pandemic and its public health implications. [Commentary]. J Translational Med. January 20, 2004; 2(3):1-4. http://www.translational-medicine.com/content/2/1/3. Accessed November 18, 2005.

Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the US. JAMA. 2001;286(10):1195-200. PMID: 11559264 DOI: 10.1001/jama.286.10.1195.

More than Six Authors
McGlynn EA, M.Asch S, Adams J, et al. The quality of health care delivered to adults in the United States. N Engl J Med. 2003;348(26):2635-45. PMID: 12826639 DOI: 10.1056/NEJMsa022615.

Jasul Jr. GV, Paz-Pacheco E, Jimeno CA, et al. AFES A.S.-O.N.E.: ASEAN Survey Of Needs in Endocrinology in the time of the COVID-19 pandemic. J AFES Fed Endocr Soc.2020;35(1):5-13. PMID:33790494 PMCID: PMC7992306 DOI: 10/15605/jafes.035.01.10.

Authors Representing a Group
Moher D, Schulz KF, Altman D; for the CONSORT Group. The CONSORT statement: Revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA. 2001;285(15):1987-91. PMID: 11308435 DOI: 10.1001/jama.285.15.1987.

Book
Byrne, DW. Publishing your medical research paper: What they don't teach in medical school. Baltimore: Williams & Wilkins, 1998.

World Wide Web
The key and critical objectives of JAMA. http://jama.ama-assn.org/misc/aboutjama.dtl. Accessed April 4, 2007.

Tables
1. Cite all tables consecutively in the text and number them accordingly.
2. Create tables, preferably using Microsoft Excel, with one table per worksheet.
3. Tables should not be saved as image files.
4. The content of tables should include a table number (Arabic) and title in capital letters above the table, and explanatory notes and legends as well as definitions of abbreviations used below.
5. Font should be Arial Narrow size 8.
6. Each table must be self-explanatory, being a supplement rather than a duplicate of information in the text.
5. Up to a maximum of five (5) tables are allowed.

Figures and Graphs
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3. Editable figures or graphs can also be created using Microsoft Word.
4. Provide a title and brief caption for each figure or graph. Caption should not be longer than 15-20 words.
5. All identifying data of the subject/s or patient/s under study such as name or case numbers should be removed.
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1. Where appropriate, all illustrations/photographic images should be at least 600 dpi and submitted as image files (preferably as .tif, .jpeg, or .png files). All images should be checked for resolution. Right click on the image and click properties to see the resolution.
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5. Figure legends should be numbered sequentially, typed double-spaced on a separate sheet of paper. Give the meaning of all symbols and abbreviations used in the figure.
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1. Upon receipt of the manuscript, the Editor shall review the submission, check if it has met aforementioned criteria and consult with members of the Editorial Board to decide whether it shall be considered for publication or not.
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3. JAFES implements a strict double blind peer review policy. For manuscripts that are reviewed, authors can expect an initial decision within forty-five (45) days after submission. There may be instances when decisions can take longer than 45 days, in such cases, the editorial assistant shall inform the authors. The editorial decision for such manuscripts shall be one of the following: (a) acceptance without further revision, (b) acceptance with minor revisions, or (c) major manuscript revision and resubmission.
4. Accepted manuscripts are subject to editorial modifications to bring them in conformity with the style of the journal.

EDITORIAL OFFICE CONTACT INFORMATION:

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ARTICLE TYPES

Original Articles
The abstract should contain no more than 200 words with a structured format consisting of the objective/s, methodology, results and conclusion. A manuscript for original articles should not exceed 25 typewritten pages (including tables, figures, illustrations and references) or 6000 words.

Reviews
Review articles provide information on the "state of the art." JAFES encourages that reviews not only summarize current understanding of a particular topic but also describe significant gaps in the research, and current debates. The abstract should be from 50 to 75 words and should not be structured. A manuscript for reviews should not exceed 15 typewritten pages (including tables, figures, illustrations and references) or 4000 words.

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The abstract should be from 50 to 75 words and should not be structured. A manuscript for case reports or case series should not exceed 10 typewritten pages (including tables, figures, illustrations and references) or 3000 words.

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JAFES may feature articles, either as part of an issue theme, such as Summary Clinical Practice Guidelines on endocrinology from each AFES country society, or a special topic on endocrinology by an international expert or authority. The abstract should be from 50 to 75 words and should not be structured. A manuscript for feature articles should not exceed 25 typewritten pages (including tables, figures, illustrations and references) or 6000 words.

Endocrine Perspectives
JAFES may invite topic experts to publish viewpoints, opinions, and commentaries on relevant topics. A manuscript for endocrine perspectives should not exceed 10 typewritten pages (including tables, figures, illustrations and references) or 3000 words. *Not peer reviewed.

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Brief Communications are short reports intended to either extend or expound on previously published research OR present new and significant findings which may have a major impact in current practice. If the former, authors must acknowledge and cite the research which they are building upon. The abstract should be from 50 to 75 words and should not be structured. A manuscript for brief communications should not exceed 5 typewritten pages (including tables, figures, illustrations and references) or 1500 words.

Editorials
Articles that represent the scientific opinion and views of an author. Every issue of JAFES includes an Editorial by the Editor-in-Chief and may include one or two additional editorials from experts from the scientific community commenting on a particular field or issue on endocrinology. No abstract or keywords necessary.

Letters to the Editor
JAFES welcomes feedback and comments on previously published articles in the form of Letters to the Editor. No abstract or keywords necessary. A Letter to the Editor must not exceed 2 typewritten pages or 500 words.

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Special announcements may include upcoming conventions, seminars or conferences relevant to endocrinology and metabolism. The Editors shall deliberate and decide on acceptance and publication of special announcements. Please coordinate with the Editorial Coordinator for any request for special announcements.

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Authors may submit interesting, unique, rare, highly educational images from actual cases with an accompanying brief history and discussion. No abstract or keywords are necessary. The image should be at least 600 dpi. The write up should not exceed 500 words with a maximum of 10 references.

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Abstract	☐ Provide an abstract conforming with the format ☐ Structured for Original Articles: Objective/s, Methodology, Results, Conclusion ☐ Unstructured for Case Reports and Feature Articles
Keywords	☐ Provide 3-5 keywords (listed in MeSH)
Content	☐ Provide text/content in IMRAD format (Introduction, Methodology, Results and Discussion, Conclusion) ☐ Make sure all abbreviations are spelled out once (the first time they are mentioned in the text) followed by the abbreviation enclosed in parentheses; the same abbreviation may then be used subsequently ☐ Make sure all measurements and weights are in SI units ☐ If appropriate, provide information on institutional review board/ethics review committee approval ☐ Acknowledgments to individuals/groups of persons, or institution/s should be included at the end of the text just before the references; grants and subsidies from government or private institutions should also be acknowledged
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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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