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Journal of the ASEAN Federation of Endocrine Societies

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ORIGINAL ARTICLES

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TABLE OF CONTENTS

Editorial

Going PubMed, Writing Together! **4**
Elizabeth Paz-Pacheco

ORIGINAL ARTICLES

The Relationship Between Admission Insulin Resistance Index (AIRI) and In-Hospital Outcome in Non-Diabetic Acute Coronary Syndrome **7**
Jorianto Muntari, Husaini Umar, Pendrik Tandean, Syakib Bakri, Himawan Sanusi, Nur Ahmad Tabri, Arifin Seweng

The Association Between Serum 25-hydroxyvitamin D and Glycemic Control in Patients with Diabetes Mellitus: A Single-Center Retrospective Study **13**
Mariel Enverga, Maria Jocelyn Isidro, Nerissa Ang-Golangco

Real-World Use of Once-Weekly Semaglutide in Thai Patients with Type 2 Diabetes Mellitus in a Private Hospital Setting **21**
Yotsapon Thewjitcharoen, Nalin Yenseung, Siriwan Butadej, Soontaree Nakasatien, Phawinpon Chotwanvirat, Waralee Chatchomchuan, Ekgaluck Wanothayaroj, Sirinate Krittiyawong, Thep Himathongkam

Obesity Awareness and its Relationship to Sociodemographic Characteristics of Filipino Adults: A Survey among Work-From-Home Employees in Metro Cebu, Philippines **29**
Jan Louie Gatillo, Ariane Corpuz, Theresa Leyco, Alexphil Ponce

Initiating or Switching to Insulin Degludec/Insulin Aspart in Adults with Type 2 Diabetes in Malaysia: Results from a Prospective, Non-Interventional Real-World Study **37**
Mafauzy Mohamed, Siang Chin Lim, Malik Mumtaz, Shweta Uppal, Deepak Mukherjee, Mohamed Saiful Mohd Kassim, Shalini Sreedharan, Amudha Murugan Doraiswamy, Kuck Meng Chong, Lu Yu Tat, Sudzilla Binti Nordin, Jeshen Lau Hui Giek, Zanariah Hussein, Khalid Abdul Kadir, Bik Kui Lau, Siew Pheng Chan

The Potential Relationship Between Serum Irisin Concentration with Inflammatory Cytokines, Oxidative Stress Biomarkers, Glycemic Indices and Lipid Profiles in Obese Patients with Type 2 Diabetes Mellitus: A Pilot Study **45**
Yaser Khajebishak, Amir Hossein Faghfouri, Ali Soleimani, Said Peyrovi, Laleh Payahoo

The Prevalence of Advanced Liver Fibrosis among Patients with Type 2 Diabetes Mellitus: A Single-Centre Experience in Penang, Malaysia **52**
Xe Hui Lee, Lisa Mohamed Nor, Choon Seong Ang, Toh Peng Yeow, Shueh Lin Lim

The Role of Triglyceride-Glucose Index in the Prediction of the Development of Hypertension – Findings from a Community Cohort in Singapore **62**
Jonathon Khoo, Serena Low, Bastari Irwan, Justin Tang, Chee Fang Sum, Tavintharan Subramaniam, Su Chi Lim

Clinicodemographic Profile and Outcomes of Type 2 Diabetes Mellitus in the Indonesian Cohort of DISCOVER: A 3-Year Prospective Cohort Study **68**
Djoko Wahono Soeatmadji, Rulli Rosandi, Made Ratna Saraswati, Roy Panusunan Sibarani, Widya Oktaviana Tarigan

Patient Characteristics, Disease Burden, Treatment Patterns and Outcomes in Patients with Acromegaly: Real-World Evidence from the Malaysian Acromegaly Registry 75
Mohamed Badrulnizam Long Bidin, Abdul Mueed Khan, Florence Hui Sieng Tan, Nor Azizah Aziz, Norhaliza Mohd Ali, Nor Azmi Kamaruddin, Shireene Vethakkan, Balraj Sethi, Zanariah Hussein

Association of Vitamin D levels on the Clinical Outcomes of Patients Hospitalized for COVID-19 in a Tertiary Hospital 81
Margarita Katrina Amor Tan, Rebecca Lim Alba, Kingbherly Li

Critical Illness-Related Corticosteroid Insufficiency (CIRCI) among Patients with COVID-19 at a Tertiary Hospital: Clinical Characteristics and Outcomes 90
Anna Elvira Arcellana, Kenneth Wilson Lim, Marlon Arcegono, Cecilia Jimeno

REVIEW ARTICLE

Incidence, Recurrence and Mortality among Filipinos with Differentiated Thyroid Cancer: A Systematic Review 100
Mari Des San Juan, Elizabeth Paz-Pacheco

CASE REPORTS

Swyer Syndrome Presenting as Dysgerminoma: A Case Report 108
Silima Tarenia, Sujaya Chattopadhyay, Niladri Das, Deep Hathi, Arjun Baidya, Puranjoy Chakrabarty, Nilanjan Sengupta, Soumik Goswami

Turner Syndrome and Neurofibromatosis 1: Rare Co-Existence with Important Clinical Implications 114
Sunetra Mondal, Neha Agrawal, Subhankar Chowdhury

Recurrent Desmoid Fibromatosis of the Thyroid Gland: A Diagnostic Challenge 120
Brijesh Kumar Singh, Sunil Chumber, Rathore Yashwant, Shipra Agarwal Shipra, Sameer Rastogi, Surabhi Vyas

New-onset Thyroid Eye Disease after COVID-19 Vaccination in a Radioactive Iodine-Treated Graves' Disease Patient: A Case Report and Literature Review 125
Jamie Hong Im Teoh, Norlaila Mustafa, Norasyikin Wahab

IMAGES IN ENDOCRINOLOGY

Multiple Xanthoma Tuberosum in a Case of Familial Homozygous Hypercholesterolemia 134
Pankaj Singhania, Pritam Biswas, Abhranil Dhar

Panhypopituitarism and Bifid Uvula 136
Pankaj Singhania, Aditya Deshpandeap

Disclosure of Editorial Conflict of Interest 138
Instructions to Authors 140
Cover Letter 146
Author Form 147
ICMJE Form for Disclosure of Potential Conflicts of Interest 150
Patient Consent Form 152
Peer Reviewers 153

Going PubMed, Writing Together!

"If you want to go fast, walk alone; if you want to go far, walk together." — African proverb

After 12 years of advocating, inviting, writing, reviewing, and publishing high-quality endocrinology research for Southeast Asia, we are very pleased to announce that the Journal of the ASEAN Federation of Endocrine Societies has been accepted for indexing in MEDLINE/PubMed, one of the highly regarded and widely used biomedical databases in the world.

As the official journal of the ASEAN Federation of Endocrine Societies, JAFES has a unique role in promoting endocrine research and practice in the ASEAN region. With its inclusion in MEDLINE/PubMed, the journal is now poised to make an even greater contribution both regionally and globally.

MEDLINE/PubMed is maintained by the United States National Library of Medicine (NLM). It provides access to over 30 million citations from more than 5,600 biomedical journals that meet strict criteria for scientific rigor, technical standards, and editorial excellence, including some of the most prominent and respected publications in the field. With its inclusion in MEDLINE/PubMed, along with prior indexing in PubMed Central, Scopus, Web of Science (WoS), Western Pacific Region Index Medicus (WPRIM), and Directory of Open Access Journals (DOAJ), JAFES will now have even greater visibility and impact, reaching a wider audience of researchers and clinicians worldwide.

The last 12 years have truly been seasons of grace and gratitude. We congratulate the authors, reviewers, editorial team, advisers, and the AFES societies on this remarkable achievement. We also extend our thanks to the MEDLINE/PubMed team for their careful evaluation and selection of JAFES.

It is our hope that this new development will further inspire and motivate our community of endocrine experts in the Southeast Asian region to continue pushing the boundaries of knowledge and contributing to the advancement of endocrinology. The next set of goals of JAFES shall focus on sustainability, continued growth, futures thinking and foresight, and measuring the impact of the journal in the years to come.

Elizabeth Paz-Pacheco
Editor-in-Chief

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After 12 years of publishing high quality endocrinology research for Southeast Asia, we are very pleased to announce that the

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ORIGINAL ARTICLE

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The Relationship Between Admission Insulin Resistance Index (AIRI) and In-Hospital Outcome in Non-Diabetic Acute Coronary Syndrome

Jorianto Muntari,[1] Husaini Umar,[1] Pendrik Tandean,[1] Syakib Bakri,[1] Himawan Sanusi,[1] Nur Ahmad Tabri,[1] Arifin Seweng[2]

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Abstract

Background. Acute coronary syndrome (ACS) is a major cardiovascular problem due to its high hospitalization and mortality rates. One of the risk factors for atherosclerosis that leads to ACS is insulin resistance (IR) which plays a role in the pathogenesis and development of cardiovascular events. This study aims to determine the relationship between IR and in-hospital outcomes in non-diabetic patients with ACS.

Methodology. This was a cohort study conducted from January-June 2021. Insulin resistance was assessed using the Admission insulin resistance index (AIRI). This measurement was performed once during the patient's admission, and then the outcome was observed during hospitalization. The observed in-hospital outcomes were composite outcomes; namely, heart failure, arrhythmia, cardiogenic shock, and death. The statistical tests used were ANOVA, independent T and Chi-Square tests. Statistical test results were considered significant if $p<0.05$.

Results. This study included 60 subjects (51 males and 9 females). Analysis revealed that AIRI was higher in patients with composite outcomes (mean 9.97 ± 4.08) than in patients without composite outcomes (mean 7.71 ± 4.06) ($p<0.05$); AIRI was higher in patients with heart failure (mean 10.72 ± 3.83) than in patients without heart failure (mean 7.25 ± 3.84) ($p<0.001$). Patients with IR had a higher rate of heart failure complications [OR 5.5 95% CI (1.56-19.38) ($p=0.005$)].

Conclusion. There is an association between AIRI and composite outcomes. Patients with IR have 5.5 times the risk of developing heart failure.

Key words: insulin resistance, acute coronary syndrome, in-hospital outcome, AIRI

INTRODUCTION

Insulin resistance (IR) pertains to impaired insulin sensitivity in maintaining plasma glucose concentrations which causes compensatory hyperinsulinemia.[1] It plays a vital role in the pathogenesis and development of cardiovascular events. Thus, fasting glycemia, post-prandial glycemia, and insulin levels can be positively correlated with new cardiac complications in ACS patients.[2]

Atherosclerosis is the main driver of clinical manifestations such as transient ischemic attack, ischemic stroke, peripheral arterial disease, angina pectoris, acute myocardial infarction, heart failure, arrhythmias, and sudden cardiac death.[2] Various studies have proven the relationship between IR and vascular disease and include IR as a new risk factor for atherosclerosis.[3,4] Insulin resistance causes atherosclerosis either directly or indirectly. Directly, IR disrupts the intracellular phosphorylase pathways PI-3-kinase and Akt, resulting in a decrease in nitric oxide (NO) production,and increasing the activation of the MAP kinase pathway, which causes smooth muscle cell proliferation and prothrombic conditions.[5] Indirectly, IR facilitates the occurrence of hyperlipidemia, hypertension, and diabetes, components of the metabolic syndrome that increase the risk of atherosclerosis which is the trigger of ACS.[3]

Admission insulin resistance index (AIRI) is an insulin resistance test measuring insulin levels at admission [insulin at admission (μIU/ml) x plasma glucose at admission (mmol/L)/22.5].[6] This has a correlation with the insulin resistance syndrome and can be used as a poor prognostic predictor in patients with ACS.[7]

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Several studies show that AIRI can be used as a prognostic tool for patients with myocardial infarction. Stubbs et al., concluded that AIRI in patients with myocardial infarction predicts poor outcomes and is superior to glucose measurement at admission.[7]

Içli et al., found higher AIRI values in patients with acute myocardial infarction than in unstable angina pectoris (7.2 ± 5.3 versus 5.2 ± 4.4, p<0.01). Admission insulin resistance index was significantly correlated with heart failure (r=0.42, p<0.0001), atrial fibrillation (r=0.35, p=0.002) and reinfarction (r=0.23, p=0.04) in patients with acute myocardial infarction (AMI).[8] Sanjuan et al., in a study of 518 patients with AMI with or without diabetes mellitus found that the mortality rate increased with increasing IR values, from 3% at IR values <2 to 18% at IR values >3. Admission insulin resistance index is a simple measure to identify IR states. The presence of IR in ACS has a role in identifying the extent of coronary vessel affectation in non-diabetic patients. [9]

This study aimed to observe the relationship between AIRI and in-hospital outcomes in non-diabetic patients with ACS. The academic benefit of the study is that the results can be serve as reference for further research on the topic.

METHODOLOGY

Study design

The design was a prospective cohort study. Insulin resistance was assessed using the admission insulin resistance index (AIRI). This measurement was performed upon the patient's admission, and the outcome was observed during hospitalization. The observed in-hospital outcomes were composite outcomes; namely, heart failure, arrhythmia, cardiogenic shock, and death.

Research subject and sample size

Subjects in this study were patients who met the inclusion criteria: aged >18 years old who had a diagnosis of acute coronary syndrome [unstable angina pectoris (UAP), non-ST elevation myocardial infarction (NSTEMI), and ST elevation myocardial infarction (STEMI)] at the time of admission, with no history of diabetes mellitus or heart disease, and who were willing to participate in the study.

The estimated number of samples required in this study was calculated based on the formula:

$$n = \frac{\left(Z_{1-\alpha/2}\sqrt{(1+k)\bar{\lambda}^2} + Z_{1-\beta}\sqrt{k\lambda_1^2+\lambda_2^2}\right)^2}{k\left(\lambda_1-\lambda_2\right)^2}$$

Based on this formula, the minimum sample size is 20 participants.

Data collection

Data was collected from the Emergency Department of the Dr. Wahidin Sudirohusodo Hospital from January-June 2021. History taking, physical examination, ECG, and troponin I examinations were used to diagnose ACS.

The subjects' venous blood were taken and blood glucoses were checked by the hexokinase-glucose-6-phosphate dehydrogenase method, which was expressed in mg/dL units, and converted to mmol/L units using the formula: blood glucose (mmol/L) = admission blood glucose (mg/dL)/18. The insulin level was checked using the ELISA kit 96T brand Demeditec and expressed in IU/L; then, the results were incorporated into the formula AIRI = random blood glucose (mmol/L) x random insulin (μIU/L) / 22.5.

Admission insulin resistance index is an insulin resistance test that does not require fasting insulin. It is obtained by checking insulin and plasma glucose at the time of admission or at any time. Although its sensitivity and specificity are still unknown, the AIRI value has a significant correlation with HOMA-IR and the insulin resistance syndrome.[7]

The gold standard examination for assessing IR is the hyperinsulinemic euglycemic clamp (HEC). Unfortunately, this examination is invasive, entails high costs, and takes a long time, so is impractical in the clinical setting. Another test that is relatively inexpensive and is easier to perform is the homeostasis model assessment-insulin resistance (HOMA-IR), which involves checking fasting insulin and plasma glucose, then entering these values into the formula: fasting insulin (μIU/ml) x glucose plasma (mmol/L)/22.5.[10–12] However, there is currently no standardized HOMA-IR cut-off for determining insulin resistance, and its utility is limited because it requires both glucose and fasting insulin.

As with HOMA-IR, there is no AIRI cut-off value to diagnose insulin resistance. To assess whether the AIRI taken as a criterion for insulin resistance can reflect variations in the AIRI value of the population studied, we classified the AIRI values of the entire study population into tertiles, with these values: tertile-1 (<6.73), tertile-2 (6.73-10.84), and tertile-3 (>10.84) values. Tertile-3 is considered as AIRI above the normal limit; therefore, the value of 10.84 was set as the threshold for insulin resistance.

The HbA1C level was obtained using chromatography with the Konelab Prime 60 tool and expressed as a percentage (%). Subjects with HbA1c levels greater than or equal to 6.5% were excluded from the study.

After the examination, the patient was observed for in-hospital outcomes, specifically arrhythmia, heart failure, cardiogenic shock and death.

In this study, we used a composite outcome which refers to an outcome consisting of two or more component outcomes. Patients who have experienced one of the events defined

by the component categorized as a composite outcome. The main benefits of utilizing component outcomes include increased statistical efficiency, the potential to increase overall event rates when individual event rates are low, and the improved resource efficiency of trials.[13]

The following were considered arrhythmias in this study: atrial flutter, atrial fibrillation, supraventricular tachycardia, ventricular tachycardia, ventricular fibrillation, and AV block grade 2-3.

The forms of heart failure considered in the outcomes are: heart failure with preserved ejection fraction (HFpEF), heart failure with mid-range ejection fraction (HFmrEF), and heart failure with reduced ejection fraction (HFrEF).[14]

The criteria for diagnosing cardiogenic shock according to the KAMIR-NIH (2018) guidelines are systolic blood pressure (SBP) <90 mmHg for >30 minutes or the use of supportive interventions to maintain SBP >90 mmHg, evidence of end-organ damage (impaired mental status, urine output <30 mL/hour, or the presence of cold extremities).[15]

Table 1. Data variable description

Characteristics	N (60)	%
Sex		
Male	51	85.0
Female	9	15.0
Diagnosis		
UAP	9	15.0
NSTEMI	27	45.0
STEMI	24	40.0
Thrombolytics		
Yes	7	11.7
No	53	88.3
Primary Cutaneous Intervention		
Yes	22	36.7
No	38	63.3
Complication		
Yes	40	66.7
No	20	33.3
Arrhythmias		
Yes	9	15.0
No	51	85.0
Heart Failure		
Yes	34	56.7
No	26	43.3
Cardiogenic Shock		
Yes	7	11.7
No	53	88.3
Death		
Yes	9	8.8
No	51	91.2

Data analysis

Data analysis was performed using SPSS version 25. The descriptive method aims to obtain general information about the research sample. Mean values, standard deviation (SD) and the frequency distribution of the sample were obtained as descriptive statistics. The statistical tests that we used were ANOVA, independent T-test, and Chi-Square tests. Statistical test results were considered as significant results if $p<0.05$.

In this study, a normality test was carried out on the AIRI value with Kolmogorov-Smirnov obtained $p>0.05$, which means that the AIRI value is normally distributed; therefore, the statistical test used is a parametric test. Subsequently, the mean AIRI was tested using ANOVA for variables consisting of more than 2 groups, while the independent T-test was used for variables made up of 2 groups.

Research permission and ethical approval

Before conducting the research, an ethical clearance approval from the Ethics Committee for Biomedical Research on Humans, Faculty of Medicine, Hasanuddin University, Makassar was obtained. The research ethics approval recommendation number is 799/UN4.6.4.5.31/PP36/2020.

RESULTS

Subject characteristics

Data analysis was conducted on 60 subjects (51 males and 9 females), ages 30-79 years old (55.98 ± 12.39 years old). In this study, the average serum insulin level was 27.68 ± 13.83 mIU/L, the average blood glucose was 8.07 ± 3.32 mmol/L, and the AIRI index was 9.22 ± 4.18 (Table 1). To date, there is no set cutoff of the AIRI value to confirm the presence of IR; therefore, in this study, we classified the AIRI values based on tertiles: tertile-1 (<6.73), tertile-2 (6.73-10.84), and tertile-3 (>10.84) values. Tertile-3 is considered as AIRI above the normal limit; thus, the value of 10.84 was set as the threshold for insulin resistance in this study.

In this study, 7 patients received thrombolytics and 22 received primary cutaneous intervention. All patients received dual antiplatelet and anticoagulant therapy. Patients with UAP and NSTEMI were subjected to individual risk stratification. For those deemed to be at

Table 2. Subject characteristics at the Emergency Department, Dr. Wahidin Sudirohusodo Hospital, from January-June 2021

	N	Minimum	Maximum	Mean	Std. deviation
Age (year)	60	30.00	79.00	55.9833	12.39258
Insulin Serum (µIU/ml)	60	10.04	77.61	27.6805	13.83629
GDS (mmol/L)	60	3.89	21.39	8.0796	3.32095
AIRI	60	2.03	24.32	9.2244	4.18206

high and very high-risk, coronary angiography and optimal revascularization were performed. Patients with STEMI received immediate reperfusion therapy, either through PCI or fibrinolysis (Table 2).

Analysis of the relationship between AIRI and the presentation of non-diabetic patients with ACS

The ANOVA test was used to analyze the relationship between AIRI and the presentation of ACS by comparing the mean AIRI values in UAP, NSTEMI, and STEMI. The results of the analysis showed no relationship between AIRI and the different types of ACS (p>0.05) (Table 3).

Analysis of the relationship between AIRI with composite outcomes and in-hospital outcomes in non-diabetic patients with ACS

The relationship between AIRI and composite outcomes during the hospitalization was analyzed. In the group with composite outcomes, the mean AIRI value was 9.97 ± 4.08 higher than in the group without composite outcomes 7.71 ± 4.06, which showed a significant relationship between AIRI and composite outcomes (p=0.047) (Table 3).

The relationship between AIRI and in-hospital outcomes was analyzed by stratifying the mean AIRI values associated with the percentage of in-hospital outcomes, such as heart failure, arrhythmias, cardiogenic shock, and death. Our results did not find any significant difference in AIRI value with arrhythmia, cardiogenic shock, and death (p>0.05), but we found a significant relationship between AIRI value and heart failure (p 0.001). In Table 3, the mean AIRI value in patients with ACS and heart failure is 10.72 ± 3.83, which is higher than in patients with ACS but without heart failure, which is 7.25 ± 3.84.

Table 3. Relationship between mean AIRI and variables

Variable	N	Mean AIRI (SD)	p*
Diagnosis			
UAP	9	9.33 (4.27)	0.942**
NSTEMI	27	9.01 (4.72)	
STEMI	24	9.41 (3.63)	
Composite outcome			0.047*
Yes	40	9.97 (4.08)	
No	20	7.71 (4.06)	
Heart Failure			0.001*
Yes	34	10.72 (3.83)	
No	26	7.25 (3.84)	
Arrhythmias			0.925*
Yes	9	9.24 (4.17)	
No	51	9.10 (4.45)	
Cardiogenic Shock			0.765*
Yes	7	9.67 (4.11)	
No	53	9.16 (4.22)	
Death			0.056*
Yes	9	11.67 (5.47)	
No	51	8.79 (3.81)	

*Independent T-test, **Annova Test

The correlation between IR and heart failure was assessed using the Chi-Square and Cochran's & Mantel-Haenszel tests, which revealed a higher rate of heart failure outcomes in those with insulin resistance (Table 4). Patients who had insulin resistance also had an increased rate of heart failure complications. [OR 5.5 CI 95% (1.56-19.38) (p=0.005)].

DISCUSSION

Our study demonstrated that the group with composite outcomes had a higher AIRI value than the group without composite outcomes. The mean AIRI value of 9.97 ± 4.08 was higher than the average AIRI in the group without the composite outcomes at 7.71 ± 4.06. This points to a significant relationship between AIRI and the presence of composite outcomes (p<0.05) (Table 3). This result is similar to that of several studies which found an association between IR and cardiovascular complications.[6,16]

A cross-sectional study by Refaie et al., on 120 non-diabetic patients showed a linear relationship between AIRI value and the number of coronary artery involvement, suggesting a role of AIRI in identifying the extent of coronary artery involvement in ACS.[6] Likewise, a cohort study by Yun et al., of 98 non-diabetic patients who underwent elective coronary angiography found that a HOMA-IR of ≥2.6, was significantly associated with adverse cardiac events (MACE) in 30 days, with a rate 27.8% higher than in those with HOMA -IR <2.6, i.e., 2.4% (p=0.008).[16]

On the other hand, the results of our study differ from that of the study of Salehiomran and Jafari (2009), which found no significant difference between the mean AIRI in patients with complications (7.9 ± 9.1) and without complications (8.7 ± 8.8).[17]

In this study, there was a correlation between insulin resistance reflected by an elevated AIRI and heart failure, but there was no correlation between insulin resistance and arrhythmias, cardiogenic shock, and death. The results of this study are similar to a study by Içli et al., in 160 non-diabetic patients, where 72 of the 160 patients

Table 4. Relationship between insulin resistance and heart failure outcome

			Heart Failure		Total	p*
			Yes	No		OR** (CI 95%)
Insulin Resistance	Yes	N	17	4	21	
		%	81%	19%	100%	0.005
	No	N	17	22	39	5.5 (1.56-19.38)
		%	43.6%	56.4%	100%	
Total		N	34	26	60	
		%	56.7%	43.3%	100%	

*Chi-Square Test, **Cochran's and Mantel-Haenszel Test

were diagnosed with acute myocardial infarction in the Coronary Intensive Care Unit. Patients were then followed up for the development of heart failure, atrial fibrillation, life-threatening ventricular arrhythmias, atrioventricular block, reinfarction, and death. Admission insulin resistance index was found to be significantly correlated with heart failure (r=0.42, p<0.0001), atrial fibrillation (r=0.35, p=0.002) and reinfarction (r=0.23, p=0.04).[8]

Insulin resistance is closely related to heart failure and is the main factor that drives heart failure both directly and indirectly.[18] Directly, IR brings about changes in intracellular metabolism, such as a decrease in intracellular glycogen and impaired glucose delivery to ischemic myocytes, thereby exacerbating the injury from infarction. Acute myocardial infarction results in cardiac dysfunction.[7,19] Indirectly, IR causes atherosclerosis which is the forerunner of acute myocardial infarction. Reduced coronary blood flow causes myocardial ischemia, where in the oxygen supply of the heart stops for approximately 20 minutes, leading to myocardium necrosis and pump failure that ultimately cause heart failure.[5,18-20]

In this study, AIRI was not correlated with arrhythmias, in contrast to the study by Sanjuan et al., which found that 20% (254/1258) of patients with acute myocardial infarction experienced high-risk ventricular tachyarrhythmias during treatment and the mortality rate (115/1,258) was higher in patients with high-risk ventricular tachyarrhythmias.[21] The pathogenic mechanism of arrhythmias due to ACS is multifactorial, including ischemia, hemodynamic and electrolyte disturbances, reentry rhythms, and automatization disorders.[22] In our study, no correlation was observed. The presence of confounding factors such as hemodynamic and electrolyte disturbances may have contributed to the lack of correlation seen in this cohort study.

The correlation between AIRI and mortality was also assessed in this study using the independent T-test and no correlation between AIRI and mortality (p > 0.05) was seen. It is consistent with the findings by Caccamo et al., which found that mortality did not appear to be significantly associated with IR (p=0.07).[2] Pathophysiological mechanisms driving the association between IR, hyperglycemia, and death in patients with acute myocardial infarction are not fully understood.[23,24]

Many studies have used the AIRI value as a predictor of outcome in ACS patients. A meta-analysis by Gast et al., with 516,325 participants from 65 studies, demonstrated that IR is a predictor of cardiovascular events.[25] Likewise, a prospective study, Insulin Resistance Atherosclerosis Study (IRAS) with 2938 patients found that IR is a significant risk factor for cardiovascular disease.[19]

Atherosclerosis is the main driver of clinical manifestations and a major cause of ischemic cardiac events such as angina pectoris, acute myocardial infarction, heart failure, arrhythmias, and sudden cardiac death. Recent data indicate that IR plays a significant role both in the pathogenesis of the metabolic syndrome and in the prediction of cardiovascular events, so that fasting glycemia, post-prandial glycemia, and insulin levels have a positive correlation with the incidence of new heart disease in ACS patients.[2,8]

Admission insulin resistance index can be used at the early stage as a predictor in high-risk patients with ACS. The AIRI value is a practical parameter that is easy to calculate and is an independent risk factor that can predict the prognosis of patients with ACS.[12] In addition, the AIRI value also has a better predictive value than blood glucose for patients with ACS.[7]

CONCLUSION

There is a relationship between the AIRI and the composite outcome of ACS which is heart failure. However, there is no correlation between AIRI and arrhythmias, cardiogenic shock, and death. Patients with IR have 5.5 times the risk of heart failure. There is no difference in AIRI value among the different types of ACS (UAP, NSTEMI, and STEMI).

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

CRediT Author Statement
JM: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Writing - original draft preparation, Supervision, Funding acquisition; **HU:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing - review and editing, Supervision, Funding acquisition; **PT:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing - review and editing, Supervision, Funding acquisition; **SB:** Conceptualization, Methodology, Resources, Writing - original draft preparation, Supervision, Project administration, Funding acquisition**; HS:** Conceptualization, Validation, Resources, Writing - original draft preparation, Visualization, Supervision, Project administration, Funding acquisition; **NAT:** Conceptualization, Validation, Resources, Writing - review and editing, Visualization, Supervision, Project administration, Funding acquisition; **AS**: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Supervision, Funding acquisition.

Author Disclosure
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ORIGINAL ARTICLE

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The Association Between Serum 25-hydroxyvitamin D and Glycemic Control in Patients With Diabetes Mellitus: A Single-Center Retrospective Study*

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Abstract

Objective. To determine the association between serum 25-hydroxyvitamin D (25(OH)D) and measures of glycemic control, hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG), in adult patients with diabetes mellitus.

Methodology. This is an analytical cross-sectional study of 270 patients with diabetes admitted to a tertiary hospital. Serum 25(OH)D levels were categorized as follows: sufficient (>30 ng/mL), insufficient (20 to 30 ng/mL), and deficient (<20 ng/mL). The correlation of HbA1c and FPG with serum 25(OH)D and other variables was determined using Spearman's rho (ρ) coefficient. The risk factors associated with HbA1c ≥7% and FPG ≥126 mg/dL were determined using logistic regression analysis to generate crude and adjusted odds ratios. The null hypothesis was rejected at 0.05 α-level of significance.

Results. The median serum 25(OH)D was 18.92 (range 3.56–56.3) ng/mL. Ninety percent (245 patients) had vitamin D levels below 30 ng/mL. This study showed that vitamin D level is significantly but weakly correlated with patient's age (ρ=0.339) and duration of diabetes (ρ=0.147), whereas it had inverse correlations with BMI (ρ=-0.134), HbA1c (ρ=-0.261), and FPG (ρ=-0.198).

Conclusion. In this study, we found a possible association between vitamin D levels and measures of glycemic control among this group of adult Filipino patients with diabetes mellitus, but further investigations in other cohorts of individuals with diabetes are needed.

Key words: Vitamin D, serum 25(OH)D, diabetes mellitus, glycemic control

INTRODUCTION

Diabetes mellitus is a complex and progressive metabolic disease. Its classification includes type 1 diabetes which involves immune-mediated destruction of the pancreatic β-cell, and type 2 diabetes characterized by relative insulin deficiency, pancreatic β-cell dysfunction, and peripheral resistance.[1] Diabetes mellitus has been a global health concern affecting millions of individuals, requiring continuous medical care with risk-reduction strategies beyond glycemic control.

Numerous data suggests that vitamin D has a pivotal role in regulating insulin secretion, insulin signaling, and improvement of insulin resistance by mediating the regulation of intracellular calcium levels.[2-4] Various cellular mechanisms in diabetes mellitus influence the metabolic signaling cascades, creating a causal link between metabolic stress and systemic inflammation.[5] Vitamin D indirectly serves an anti-inflammatory role by its effects on the cells of the immune system that secrete the pro-inflammatory cytokines which contribute to insulin resistance and autoimmune-mediated destruction of the β-cells.[4,6]

The status of vitamin D is determined by measuring serum 25(OH)D.[4,7] Studies suggest that an inverse relationship exists between vitamin D levels and measures of glycemic control, such as HbA1c and FPG.[2] In addition, a low level of vitamin D is associated with increased incidences of abdominal obesity, cerebrovascular diseases, myocardial infarction, and metabolic syndrome.[3,4]

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Vitamin D deficiency seems prevalent in Asia, with more than 50% of the population having vitamin D deficiency, while approximately 75% have insufficiency.[3] In a study of South Asian women, patients with insulin resistance and vitamin D deficiency were treated with vitamin D, 4000 IU/day. In this study, participants who achieved serum 25(OH)D above 32 ng/mL showed significant improvement in insulin sensitivity, thereby improving glycemic control.[3]

The 8th National Nutrition Survey (NNS) in 2013 showed that Filipino adults had a high prevalence of low vitamin D levels; vitamin D deficiency and insufficiency had a combined prevalence of 48.7% and were predominant in the National Capital Region (NCR).[8] This implies that Filipinos are at risk for hypovitaminosis D. Sufficient serum 25(OH)D level has been associated with optimal bone mineral density, muscle strength, and prevention of fractures.[9-11] Screening for vitamin D status is important to public health.

Due to the link between vitamin D levels and glucose homeostasis, screening for vitamin D deficiency and insufficiency in individuals with elevated HbA1c should be considered.[2,4,7] Moreover, supplementing low vitamin D levels may be an adjunctive treatment in managing diabetes, but further studies are still needed.[12] This study aims to determine the association between serum 25-hydroxyvitamin D [25(OH)D] and measures of glycemic control (HbA1c and FPG) in adult patients with diabetes mellitus.

METHODOLOGY

This study was approved by the Institutional Review Board (IRB) of the Makati Medical Center. The authors adhered to the ethical considerations set out in relevant guidelines, including the Declaration of Helsinki and the National Ethics Guidelines for Health Research and Data Privacy Act of 2012. The investigators completed the Good Clinical Practice (GCP) training on the responsible conduct of research with human data.

This cross-sectional analytical study was conducted in a private tertiary hospital in Metro Manila, Philippines. A retrospective review of the medical records of the study population from January 1, 2017 to June 30, 2021 was conducted. Convenience sampling was used for data collection.

In this study, patients were included if they had the following: (1) Serum 25(OH)D assay, HbA1c, and FPG done during admission at the study institution; (2) Normal serum total calcium or ionized calcium levels.

Patients were classified as having diabetes mellitus if they had at least one of the following criteria: (1) Diagnosed with diabetes mellitus type 1 or 2 based on medical records; (2) Use of oral and/or injectable anti-diabetic medications; (3) Previous laboratory results which included at least one of the following criteria: FPG ≥126 mg/dL; random plasma glucose ≥200 mg/dL with signs of polyuria, polydipsia or weight loss; 2-hour oral glucose tolerance test (OGTT) ≥200 mg/dL or HbA1c ≥6.5% [American Diabetes Association (ADA)].[13]

Since this is a retrospective review, the exposure to sunlight, physical activity, and dietary habits of the patients were not investigated.

The exclusion criteria were as follows: (1) Age less than 18 years old; (2) Patients with no available serum 25(OH) D assay and no serum total calcium or ionized calcium levels; (3) Pregnant or breastfeeding patients; (4) Patients with any acute or chronic blood loss, hemolytic anemia and known hemoglobin variants; (5) Patients with chronic liver disorder, chronic kidney disease or end-stage renal disease; (6) Patients with bone-mineral disorders such as but not limited to hypercalcemia, secondary osteoporosis, and hyperparathyroidism; (7) Patients taking calcium and vitamin D supplements before admission; (8) Intake of any other medications that may interfere with vitamin D metabolism such as glucocorticoids, antiestrogen, antiresorptive medications, and bisphosphonates; (9) Patients who underwent removal of any of the parathyroid glands.

Sample size

The sample size was computed using an online calculator from the University of California San Francisco, Clinical and Translational Science Institute (http://www.sample-size.net/correlation-sample-size/). Based on the study of Alkhatatbeh et al., the correlation of 25(OH)D levels with HbA1c and FPG levels was -0.23 and -0.17, respectively.[1] Assuming that the same results were obtained and using the power of 80% at 95% confidence level and accounting for 10% attrition rate, results showed that the sample size required for determining the correlation of serum 25(OH) D levels with HbA1c and FPG levels were 146 and 269, respectively. The final sample size was 299. A total of 1349 records were reviewed in this study. A total of 270 participants were included and analyzed (Figure 1).

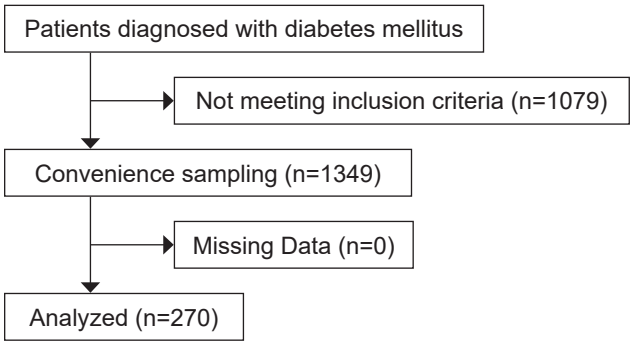

Figure 1. Flow diagram of patients included in the study.

Evaluation of vitamin D levels and glycemic control

In this study, the vitamin D status was determined by measuring the serum 25(OH)D levels. The classification of vitamin D levels was categorized as follows: sufficient (>30 ng/mL), insufficient (20 to 30 ng/mL), and deficient (less than 20 ng/mL).[4,7]

HbA1c is a glycemic target that needs to be individualized based on several factors: age, life expectancy, comorbid conditions, duration of diabetes, risk of hypoglycemia, and adherence to therapy. According to the 2021 ADA guidelines, the achievement of HbA1c levels less than the goal of 7% may be acceptable and even beneficial if it can be safely achieved without significant hypoglycemia or other treatment adverse effects.[13] However, HbA1c goals of less than 8% may be appropriate for selected patients with limited life expectancy, or when the harms of treatment outweigh the benefits.[12,13] HbA1c is an integrated measurement of fasting and post-meal blood glucose levels during the preceding 6 to 8-week period.[12] The therapeutic plan depends on the physician's judgment and the patient's preference.

The FPG correlates with mean daily plasma glucose but may not be representative of long-term glycemic control compared to HbA1c. Diabetes mellitus is diagnosed at FBS ≥126 mg/dL on two separate samples.[13] However, acute stress, illness, and infection can increase glucose production and impair its utilization, thereby increasing fasting glucose.[14]

Statistical analysis

Descriptive statistics were used to summarize the general and clinical characteristics of the participants. Shapiro-Wilk and Levene's tests were used to determine the normal distribution and homogeneity of variance of continuous variables, respectively. Continuous data which follow the normal distribution were summarized using mean and standard deviation, while non-Gaussian variables were reported as median and range. Categorical variables were reported as frequency and proportion.

Continuous variables that satisfied the dual assumptions of normal distribution and variance homogeneity were compared using an independent t-test. If both assumptions were violated, the non-parametric Kruskal-Wallis H test was used for comparison. The Chi-square test was used to compare categorical variables. If the expected percentages in the cells are less than 5%, Fisher's Exact Test was used instead.

Spearman's rho (ϱ) coefficient was used in determining the correlation of HbA1c and FPG with variables such as age, BMI, duration of diabetes, and vitamin D. According to Evans, less than 0.20 is very weak, 0.20 to 0.39 is weak, 0.40 to 0.59 is moderate, 0.60 to 0.79 is strong, and 0.80 or greater is a very strong correlation.[15] The variables associated with HbA1c ≥7% and FPG ≥126 mg/dL were determined using logistic regression analysis. The crude odds ratios (OR) and their corresponding 95% confidence intervals were estimated. Potential confounders (age, sex, BMI, hypertension, smoking history, alcohol drinking, and serum total cholesterol) were included in the multivariable model. Adjusted odds ratios and their 95% confidence intervals were reported. The null hypothesis was rejected at 0.05 α-level of significance. STATA version 15.0 (StataCorp SE, College Station, TX, USA) was used for data analysis.

RESULTS

The study included 270 adult patients with diabetes. The mean age was 57 ± 16 years with a slight female preponderance (56%) (Table 1). The median BMI was 26.7 kg/m^2, and more than a third were obese (38.5%). A majority had a diabetes duration of 1 to 10 years (60%), without a history of smoking (68%) or alcohol drinking (65%). Hypertension (66%) was the most common comorbidity, followed by dyslipidemia (35%).

The median serum 25(OH)D was 18.92 (range 3.56–56.3) ng/mL. 57% (n=155) were vitamin D deficient, 33% (n=90) were insufficient, while 10% (n=26) had sufficient vitamin D levels. Age was shown to be progressively lower with more deficient levels of vitamin D. Patients below 60 years comprised significantly more of the vitamin D deficient (66%) than insufficient (51%) patients, and more of the insufficient than sufficient (23%) patients. BMI was likewise seen to decrease with more sufficient levels of vitamin D. The median values of BMI in kg/m^2 were 27.55, 26.71, and 24.58 in the vitamin D deficient, insufficient, and sufficient groups, respectively. There were more patients with hypertension (88% vs. 60%) among the vitamin D sufficient versus deficient patients.

The median levels of HbA1c, FPG, and LDL-C among patients were elevated, whereas those for total cholesterol, triglyceride, and HDL-C were within normal ranges (Table 2). HbA1c was shown to be progressively lower with more sufficient levels of vitamin D. The median HbA1c levels were 9.76%, 7.7%, and 6.88% in the deficient, insufficient, and sufficient vitamin D groups, respectively. The median FPG was significantly higher among vitamin D deficient (173.71 mg/dL) than among insufficient (141.46 mg/dL) patients.

The vitamin D level was significantly but weakly correlated with the patient's age (ϱ=0.339) and duration of diabetes (ϱ=0.147), whereas it had inverse correlations with BMI (ϱ= -0.134), HbA1c (ϱ=-0.261), and FPG (ϱ =-0.198) (Table 3).

Age, smoking, and total cholesterol were found to be associated with elevated HbA1c, even after adjusting for covariates (Table 4). Vitamin D was crudely associated with elevated HbA1c; specifically, patients who had vitamin D deficiency were 4.484 times (95% CI 1.89 to 10.64) as likely

Table 1. Clinical and demographic profile of patients with diabetes mellitus

	All (n=270)	HbA1c <7% (n=79)	HbA1c ≥7% (n=191)	*p*
	Mean ± SD; Frequency (%); Median (Range)			
Age, years	56.19 ± 16.05	64.44 ± 15.94	52.77 ± 14.84	<0.001*
<60	154 (57.04)	29 (36.71)	125 (65.45)	
>60	116 (42.96)	50 (63.29)	66 (34.55)	
Sex				0.268†
Male	120 (44.44)	31 (39.24)	89 (46.6)	
Female	150 (55.56)	48 (60.76)	102 (53.4)	
Weight, kg [n=267]	70 (39–163)	69 (39–120)	71.5 (40–163)	0.326‡
Height, cm [n=267]	161 (120–180)	160 (143.5–179)	161.77 (120–180)	0.618‡
BMI, kg/m^2 [n=267]	26.71 (16.23–80.71)	25.74 (16.23–80.71)	27.08 (16.79–63.89)	0.928‡
Underweight	7 (2.62)	2 (2.53)	5 (2.66)	
Normal	75 (28.09)	21 (26.58)	54 (28.72)	
Overweight	81 (30.34)	24 (30.38)	57 (30.32)	
Obese I	51 (19.1)	16 (20.25)	35 (18.62)	
Obese II	19 (7.12)	4 (5.06)	15 (7.98)	
Obese III	34 (12.73)	12 (15.19)	22 (11.7)	
Blood pressure, mmHg				
Systolic	130 (79–214)	130 (90–190)	130 (79–214)	0.656‡
Diastolic	80 (50–134)	80 (60–100)	80 (50–134)	0.161‡
Smoking history				0.600†
No	183 (67.78)	55 (69.62)	128 (67.02)	
Current	33 (12.22)	11 (13.92)	22 (11.52)	
Former	54 (20)	13 (16.46)	41 (21.47)	
Alcohol drinker				0.825§
No	175 (64.81)	51 (64.56)	124 (64.92)	
Current	81 (30)	23 (29.11)	58 (30.37)	
Previous	14 (5.19)	5 (6.33)	9 (4.71)	
Comorbidities				
Diabetes mellitus type I	4 (1.48)	0 (0)	4 (2.09)	0.325§
Gestational diabetes	0 (0)	0 (0)	0 (0)	-
Hypertension	178 (65.93)	61 (77.22)	117 (61.26)	0.012†
Dyslipidemia	94 (34.94)	31 (39.24)	63 (33.16)	0.341†
Kidney disease	13 (4.81)	6 (7.59)	7 (3.66)	0.211§
Stroke or MI	43 (15.99)	17 (21.52)	26 (13.68)	0.110†
Thyroid disease	23 (8.52)	10 (12.66)	13 (6.81)	0.117†
Hypothalamic disease	0 (0)	0 (0)	0 (0)	-
Liver disease	4 (1.48)	1 (1.27)	3 (1.57)	0.999§
Inflammatory disease	4 (1.48)	3 (3.8)	1 (0.52)	0.077§
Neoplastic disease	5 (1.85)	3 (3.8)	2 (1.05)	0.151§
Others	47 (17.41)	19 (24.05)	28 (14.66)	0.064†
Years since DM diagnosis				0.452§
0–<1	52 (19.26)	11 (13.92)	41 (21.47)	
1–10	163 (60.37)	52 (65.82)	111 (58.12)	
11–20	38 (14.07)	10 (12.66)	28 (14.66)	
>20	7 (2.59)	1 (1.27)	6 (3.14)	
Unrecalled	10 (3.7)	5 (6.33)	5 (2.62)	

Statistical tests used: * - Independent t-test; † - Chi-square test; ‡ - Mann-Whitney U test; § - Fisher's exact test.

Table 2. Laboratory profile of patients with diabetes mellitus

	All (n=270)	HbA1c <7% (n=79)	HbA1c ≥7% (n=191)	*p*
	Median (Range); Frequency (%)			
25(OH)D, ng/mL	18.92 (3.56–56.3)	21.6 (5.56–56.3)	18.04 (3.56–36.4)	<0.001
Deficient (<20)	155 (57.41)	32 (40.51)	123 (64.4)	
Insufficient (20–30)	89 (32.96)	33 (41.77)	56 (29.32)	
Sufficient (>30)	26 (9.63)	14 (17.72)	12 (6.28)	
Total cholesterol, mg/dL	179.58 (75.79–516.88); [n=246]	159.67 (75.79–346.01); [n=66]	189.05 (81.19–516.88); [n=180]	0.001
HDL-C, mg/dL	40.59 (5.8–86.99); [n=246]	44.46 (14.46–73.07); [n=66]	38.47 (5.8–86.99); [n=180]	0.001
LDL-C, mg/dL	119.07 (22.42–298.04); [n=245]	92.37 (25.2–298.04); [n=66]	125.65 (22.42–272.92); [n=179]	0.005
Triglyceride, mg/dL	126.56 (23.01–790.31); [n=248]	100.45 (40.7–384.09); [n=66]	140.72 (23.01–790.31); [n=181]	<0.001
FPG, mg/dL	154.16 (12.52–366)	122 (37–263.09)	185.97 (12.52–366)	<0.001
HbA1c, %	8.71 (4.83–20.55)	6.17 (4.83–6.99)	10.16 (7–20.55)	-

Statistical test used: Mann-Whitney U test.

to have an HbA1c ≥7%. However, this association was no longer significant after adjusting for covariates (Table 4).

Using vitamin D as a continuous scale, this study found that patients with low vitamin D were 5.2 times less likely to have elevated FPG levels (crude OR 0.947, 95% CI 0.91 to 0.98, *p*=0.001). However, there was no association between vitamin D status and glycemic control after adjusting for covariates. Meanwhile, serum total cholesterol was found to be associated with elevated FPG even after adjusting for covariates (adjusted OR 1.011, 95% CI 1.005 to 1.02, *p*<0.001) (Table 5).

Table 3. Correlations of serum 25(OH)D with other patient factors

	25(OH)D (ng/mL)	
	Rho	*p*
Age, years	0.3386	<0.001
BMI, kg/m^2 [n=267]	-0.1343	0.028
Years since DM diagnosis [n=260]	0.1473	0.018
HbA1c, %	-0.2607	<0.001
FPG, mg/dL	-0.1983	0.001

DISCUSSION

The role of inflammation in the pathogenesis of diabetes mellitus and its associated metabolic disorders has been an emerging interest in its management.[16] The relationship between vitamin D levels and insulin resistance can be realized at the level of immunomodulatory processes and systemic inflammation, influencing the autoimmune pathology in type 1 diabetes and the low-grade chronic inflammation in type 2 diabetes.[6] The vitamin D receptors are expressed in different tissues, such as the adipose,

Table 4. Logistic regression analysis of the variables with HbA1c

	HbA1c ≥7%	*p*	HbA1c ≥7%	*p*
	Crude OR (95% CI)		Adjusted** OR (95% CI)	
Vitamin D, ng/mL	0.919 (0.88 to 0.95)	<0.001	-	
Vitamin D status				
Deficient	4.484 (1.89 to 10.64)	0.001	2.644 (0.94 to 7.47)	0.067
Insufficient	1.980 (0.82 to 4.79)	0.129	1.453 (0.50 to 4.21)	0.491
Sufficient	Reference (1.0)	-	Reference	-
Age	0.951 (0.93 to 0.97)	<0.001	0.952 (0.93 to 0.97)	<0.001
Male sex	1.351 (0.79 to 2.30)	0.269		
BMI	1.025 (0.99 to 1.07)	0.220		
Hypertension	0.467 (0.26 to 0.85)	0.013		
Smoking history				
No	Reference (1.0)	-	Reference (1.0)	-
Current	0.859 (0.39 to 1.89)	0.707	0.306 (0.12 to 0.77)	0.011
Former	1.355 (0.67 to 2.73)	0.394		
Alcohol drinker				
No	Reference (1.0)	-		
Current	1.037 (0.58 to 1.86)	0.902		
Previous	0.740 (0.24 to 2.32)	0.605		
Total cholesterol, mg/dL	1.008 (1.002 to 1.01)	0.004	1.006 (1.0001 to 1.01)	0.045
Adjusted R^2	-		14.84%	

**Vitamin D as a categorical predictor was forced into the final model using STATA lockterm 1 function

Table 5. Logistic regression analysis of the variables with FPG

	FPG ≥126 mg/dL	*p*	FPG ≥126 mg/dL	*p*
	Crude OR (95% CI)		Adjusted** OR (95% CI)	
Vitamin D, ng/mL	0.947 (0.91 to 0.98)	<0.001		
Vitamin D status				
Deficient	1.815 (0.74 to 4.43)	0.190	1.206 (0.42 to 3.50)	0.731
Insufficient	0.817 (0.33 to 2.04)	0.664	0.496 (0.17 to 1.49)	0.210
Sufficient	Reference (1.0)	-	Reference (1.0)	-
Age	0.977 (0.96 to 0.99)	0.008		
Male sex	0.938 (0.55 to 1.59)	0.811		
BMI	1.019 (0.98 to 1.06)	0.359		
Hypertension	0.612 (0.34 to 1.09)	0.096		
Smoking history				
No	Reference (1.0)	-		
Current	0.695 (0.32 to 1.51)	0.359		
Former	1.032 (0.52 to 2.03)	0.927		
Alcohol drinker				
No	Reference (1.0)	-		
Current	0.924 (0.52 to 1.65)	0.788		
Previous	0.519 (0.17 to 1.57)	0.246		
Total cholesterol, mg/dL	1.011 (1.005 to 1.02)	<0.001	1.011 (1.005 to 1.02)	<0.001
Adjusted R^2			8.26%	

**Vitamin D was analyzed as a categorical predictor

skeletal muscles, and pancreatic β-cells. Vitamin D has a pivotal role in regulating insulin secretion, insulin signaling, and improving insulin resistance.[3,4]

This study showed that vitamin D level is significantly but weakly correlated with the patient's age (ϱ=0.339) and duration of diabetes (ϱ=0.147), whereas it had inverse correlations with BMI (ϱ=-0.134), HbA1c (ϱ=-0.261), and FPG (ϱ=-0.198) (Table 3).

All participants were admitted and were assumed to have an acute illness; hence, some patients were expected to have higher FPG. Nevertheless, the median FPG was still significantly higher among vitamin D deficient (173.71 mg/dL) than among the insufficient (141.46 mg/dL) patients. On the other hand, HbA1c is a good clinical indicator since it reflects 2-3 months of glucose control.

In the study of Aalkhatatbeh et al., correlation analysis showed significant inverse correlations between 25(OH) D levels and HbA1c and FPG levels (r= 0.23 and 0.17, respectively, both $p<0.01$).[2] Multiple linear regression analysis revealed a significant inverse association between HbA1c and 25(OH)D levels (F=12.95, R2=0.48, $p<0.01$).[2]

Buhary et al., also detected a significant inverse association between HbA1c and 25(OH)D and observed that supplementation of vitamin D improved glycemic control by reducing HbA1c levels.[4] Ghavam et al., supports the findings of this study wherein an inverse linear relationship exists between 25(OH)D and HbA1c ($p<0.37$) and FPG ($p<0.64$).[12] The inverse correlation observed in this study for vitamin D and HbA1c in type 2 diabetic patients is similar to the findings of Salih et al., who showed that 25(OH)D level was significantly lower ($p<0.001$) for patients with poor glycemic control.[17]

As parameters for glucose control, elevated HbA1c and FPG may reflect greater insulin resistance and systemic inflammation. As part of its anti-inflammatory and immunomodulatory effects, vitamin D can influence glucose metabolism through its regulation of insulin secretion and signaling. Vitamin D deficiency can reduce intracellular calcium regulation of the expression of the insulin receptor, insulin signaling, and secretion, thereby affecting glucose levels.[18]

Our findings showed that the serum 25(OH)D level is significantly but weakly correlated with the patient's age (ϱ=0.339). Analysis by HbA1c indicated that those with poorer glycemic control were younger (mean: 53 vs. 64 years) even after adjusting for covariates, which may have influenced the levels of vitamin D in the study population. Findings were similar to Buhary et al., who found that older patients had higher vitamin D levels ($p=0.0001$).[4] Salih et al., and Yilmaz et al., did not demonstrate any significant association between age and vitamin D levels.[17,19] Salih et al., discussed that age is likely to negatively correlate with vitamin D since its production by sunlight in less efficient in older individuals. According to Gallagher, aging affects the metabolism of vitamin D and calcium through the following mechanisms: malabsorption of calcium; intestinal resistance of calcium absorption to circulating $1,25(OH)_2D$; decreased vitamin D receptors; impaired renal production of $1,25(OH)_2D$ with the age-related decline in kidney function; and reduced skin production of vitamin D.[20]

In this study, BMI was seen to decrease with more sufficient vitamin D levels, and this difference was significant. The median values of BMI in kg/m^2 were 27.55, 26.71, and 24.58 in the vitamin D deficient, insufficient, and sufficient groups, respectively. Ghavam et al., found an inverse linear relationship between vitamin D and BMI ($p<0.59$).[12] Similarly, the findings conducted by Sahli et al., showed that BMI had a highly significant effect ($p<0.001$) on vitamin D levels among patients with diabetes.[17]

Obesity has been identified as a known risk factor for vitamin D deficiency. A consequence of obesity is the impaired secretion of adipokines and systemic inflammation, which contributes to greater insulin resistance.[6,17] Higher vitamin D levels are accompanied by lower inflammatory markers, including tumor necrosis factor-α, interleukin-6, and C-reactive protein in those with inflammatory-associated diseases such as diabetes.[6] In obesity, there is increased storage of fat-soluble vitamin D in the adipose tissue and in the liver, which impairs the modulatory effects on the vitamin D receptors.[17,21,22] Obese patients are also at risk for a sedentary lifestyle which contributes to their inadequate sunlight exposure and lesser physical activity, thereby decreasing the conversion to the active form of vitamin D, $1,25(OH)_2D$.

Our findings showed a significant but weak negative correlation between the duration of diabetes (ϱ=-0.140) and 25(OH)D (ϱ=-0.198). Sahli et al., showed a significant difference between 25(OH)D levels of patients with a diabetes duration of >5 years and those with diabetes duration <5 years ($p=0.002$).[17] Ghavam et al., indicated no significant relationship between the duration of diabetes and vitamin D ($p<0.1$, r= 0.164).[12]

The retrospective nature of this investigation does not provide further insight. An area of future analysis is whether vitamin D supplementation will improve glycemic control and reduce the risk of the development of diabetes.

The anti-inflammatory and immunomodulatory effects of vitamin D may be modified by cigarette smoke since it contains harmful chemicals.[23,24] Cigarette smoking can also lower the production of the active form of vitamin D and may affect the expression of its vitamin D receptor.[23,24] Our findings showed that smoking was associated with elevated HbA1c levels, even after adjusting for covariates. Salih et al., showed that 25-hydroxyvitamin D levels are lower in smokers though the difference was not significant.[17] On the other hand, Hermann et al., showed that serum vitamin

D levels and osteocalcin were inversely related to the number of cigarettes smoked per day (r=0.11 and p<0.001; r=0.17 and p=0.04, respectively).[25]

Patients with hypovitaminosis D were younger, had higher BMI, HbA1c, and FPG levels. Adjusted associations revealed that HbA1c was progressively lower with more sufficient vitamin D levels, and the median FPG was significantly higher among vitamin D deficient patients. Our study suggests that the serum 25(OH)D levels may influence glucose homeostasis of patients with diabetes mellitus. This study supports the emerging role of vitamin D in metabolic dysregulation, pancreatic β-cell function, and inflammation in diabetes. Upreti et al., showed that oral vitamin D supplementation was associated with improved glycemic control and other metabolic parameters in diabetes mellitus.[26] However, a meta-analysis showed insufficient evidence of a beneficial effect to recommend vitamin D supplementation to improve glycemic control in patients with type 2 diabetes.[17] Further investigations are needed to validate these findings.

Limitations and recommendations of the study

Since the participants were not randomly sampled, an inherent selection bias is present in this study. Being primarily a retrospective review, confounding variables such as diet, physical activity, lifestyle, and sunlight exposure were not investigated. The authors suggest a research endeavor where additional data can be collected using interviews to document sunlight exposure, physical activity, and dietary habits.

The investigators recommend recruiting healthy patients from the outpatient clinics since the acute illness of admitted patients may have affected their metabolic state and glucose homeostasis. Another future research endeavor is to determine whether vitamin D supplementation can improve insulin sensitivity and affect glucose homeostasis. This may elucidate a causal relationship between vitamin D status, metabolic syndrome, and the microvascular and macrovascular complications of diabetes.

CONCLUSION

Our results demonstrated a weak inverse correlation between vitamin D levels and FPG and HbA1c levels. Vitamin D was also seen to be crudely associated with glycemic control, but such an association was not sustained after adjusting for covariates.

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

CRediT Author Statement
ME: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition. **MJI:** Conceptualization, Validation, Writing – review and editing, Supervision, Funding acquisition. **NSAG:** Conceptualization, Validation, Writing – review and editing, Supervision, Project administration, Funding acquisition.

Author Disclosure
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ORIGINAL ARTICLE

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Real-World Use of Once-Weekly Semaglutide in Thai Patients With Type 2 Diabetes Mellitus in a Private Hospital Setting

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Abstract

Objective. To evaluate the real-world use of once-weekly semaglutide among Thai patients with type 2 diabetes (T2DM) in a private hospital setting.

Methodology. A retrospective review of Thai patients with T2DM who have initiated semaglutide for at least 1 month between June 2020 and March 2022 at Theptarin Hospital, Bangkok, Thailand.

Results. A total of 58 patients (50% female, mean age 55.6 ± 15.9 years, with duration of diabetes 12.6 ± 10.3 years, BMI 31.5 ± 4.4 kg/m^2, baseline HbA_{1c} 7.9 ± 1.9%, with prior GLP-1 RA use 24.1%, and concomitant SGLT2i intake (41.4%) were included. During a median follow-up of 6 months, the mean serum HbA_{1c} level reduction was 1.3 ± 1.7% with weight loss of 4.7 ± 4.1 kg. The proportion of patients who achieved optimal and sustainable glycemic control (HbA_{1c} < 7.0%) increased from 43.1% to 55.8% at the last follow-up. The proportion of patients reaching both HbA_{1c} targets of <7.0% and 5% weight loss was 27.8%. No cases of pancreatitis, cancer, or progressive retinopathy were observed.

Conclusions. In this single center undertaking, it was shown that in among persons with T2DM and obesity in Thailand, semaglutide was associated with short-term glycemic control and weight loss comparable with what has been observed in randomized clinical trials and other RWE.

Key words: semaglutide, once-weekly, real-world, Thai

INTRODUCTION

Glucagon-like peptide-1 receptor agonists (GLP-1 RA) have been demonstrated by many randomized controlled trials (RCT) to improve glycemic control, and reduce body weight. It is advocated as a first-line injectable therapy in patients with type 2 diabetes mellitus (T2DM) with established atherosclerotic cardiovascular disease (ASCVD) or multiple risk factors for ASCVD in the United States.[1,2] However, the clinical characteristics of people with T2DM in Asia differ from those in Western countries. Asians have a lower BMI, higher risk of developing comorbidities, and younger age at onset of type 2 diabetes mellitus.[3,4] Therefore, real-world evidence (RWE) are required to translate the efficacy of interventions in trials to effectiveness in clinical practice across a broader spectrum of patient populations. Moreover, the cost of most of these newer agents remains prohibitive and inaccessible in low- and middle-income countries (LMICs). The RWE results might also be used for healthcare policy formation to prioritize novel treatments.[5]

Once-weekly semaglutide is a subcutaneous GLP-1 RA with the strongest effect on glycated hemoglobin (HbA_{1c}) and body weight (BW), first approved in the United States in December 2017, and became available in Thailand in June 2020. Various phase III RCTs known as the Semaglutide Unabated Sustainability in Treatment of T2DM (SUSTAIN) demonstrated superiority in its efficacy over comparators and SUSTAIN-6 trial showed a 26% lower risk of the primary composite outcome of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke than those receiving placebo.[6,7] Recently, the Semaglutide Real-world Evidence (SURE) program comprised of nine observational real-world studies investigating semaglutide initiation in routine clinical practice among European countries and Canada, also confirmed clinically significant improvements in glycemic control and reduction in BW.[8-12] Results from other RWE conducted in other countries also yielded comparable findings.[13-18]

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The unique physicochemical properties of semaglutide may contribute to the greater weight loss observed with semaglutide use compared to other GLP-1 RAs and this benefit is mostly unaffected by gastrointestinal (GI) adverse events.[19,20] Nevertheless, despite the known benefits of GLP-1 RA, long-term adherence rates are suboptimal in routine practice, potentially due to injection-related concerns and high costs.[21]

To date, there are no published real-word data on semaglutide among South-East Asians with T2DM. Therefore, this study aimed to evaluate the real-world use of once-weekly semaglutide among Thai people with T2DM in a private setting.

METHODOLOGY

This retrospective observational cohort study included all Thai people with T2DM who were given once-weekly semaglutide for at least 1 month between June 2020 and March 2022 at Theptarin Hospital, a specialized diabetes center in Bangkok, Thailand. Exclusion criteria included non-Thai patients, patients without T2DM, and duration of semaglutide usage less than 1 month. The first prescription date was the index date and all patients were followed up from then until the end of June 2022 or the last medication usage. Data were collected at baseline and at follow-up visits (3, 6 months, and at the last visit after treatment). Primary endpoints (change in serum HbA_{1c} level and BW) were assessed at baseline and at the last follow-up visit.

Secondary endpoints including change in glycemic and weight-loss targets achievement were also assessed. The subgroup analyses were conducted post-hoc. Baseline serum HbA_{1c} level (≤ 8.0%, >8.0–≤ 9.0%, and >9.0 %) and baseline body mass index (BMI) (< 30 kg/m^2, ≥30–<35 kg/m^2, and ≥35 kg/m^2), and background medication use (sulfonylurea; SU, thiazolidinedione; TZD, sodium–glucose co-transporter 2 inhibitor; SGLT2i, or insulin as background glucose-lowering medication) were chosen as basis for the subgroups. Since both GLP-1 RA and SGLT2i are newer classes of anti-diabetic medications that have shown additional cardiovascular and renal benefits, our present study also dealt with the real-world effectiveness of the combination of GLP-1 RA and SGLT2i compared with SGLT2i alone in Thai people with T2DM. Safety data included self-reported GI side effects, abdominal discomfort, documented pancreatitis, cancer, or progressive diabetic retinopathy. Severe hypoglycemic events requiring assistance of another person to actively administer carbohydrates or other resuscitative actions were also collected from medical records.

This study was approved by the Institutional Review Board committee of Theptarin Hospital (EC No.05-2020).

Statistical analysis

Demographics data were presented using descriptive statistics (mean ± standard deviation (SD) or median (interquartile range - IQR Q1, Q3) while categorical variables were summarized using counts and percentages. Baseline data and outcomes were compared between SGLT2i-treated patients and non-SGLT2i-treated patients to determine the real-world effectiveness of the combination of GLP-1 RA and SGLT2i. Normality of data was assessed using the Kolmogorov-Smirnov test. A repeated-measures Analysis of Variance (ANOVA) with a post-hoc Dunnett test comparing the follow-up time points with baseline data was performed and differences between the paired-sample proportions of those who achieved various target HbA_{1c} levels between baseline and last-visit were analyzed by McNemar test. For the subgroup analysis of background medications, the change of HbA_{1c} and BW from baseline to the last visit in each class of anti-diabetic medication compared with other background medications (mean of two independent groups) were analyzed using an unpaired *t-test* if data were normally distributed or a Mann–Whitney U test if data were not normally distributed. A $p<0.05$ was considered statistically significant. All analyses were conducted using SPSS Statistical Package, version 20 (IBM Corp., Armonk, NY, USA).

RESULTS

Baseline characteristics of the patients

During the study period, a total of 102 patients were prescribed once-weekly semaglutide injection but 44 patients were excluded for various reasons as shown in Figure 1. Treatment discontinuations within 1 month due to GI adverse events were found only in 2 patients (2.0%). Full analysis was done in the remaining 58 patients (50% female, mean age 55.6 ± 15.9 years, duration of diabetes 12.6 ± 10.3 years, baseline BW 86.7 ± 14.5 kg, BMI 31.5 ± 4.4 kg/m^2, baseline serum HbA_{1c} level 7.9 ± 1.9%, prior GLP1-RA use 24.1%, concomitant SGLT2i intake 41.4%, were included in the study with a median follow-up of 6 months (3-12 months). The clinical characteristics of the patients stratified by SGLT2i use are shown in Table 1.

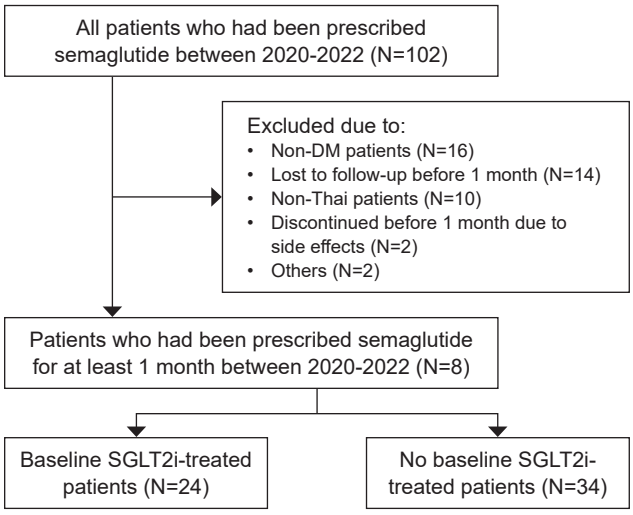

Figure 1. Flow diagram of studied patients (N=58).

Overall, 77.3% of patients were initiated on a 0.25 mg dose of semaglutide. The majority of GLP-1 RA-naïve patients were prescribed a starting semaglutide dose of 0.25 mg, whereas approximately half of the patients in the group with prior GLP-1RA use were started on a 0.5 mg or 1.0 mg dose. At the last follow-up, the majority of patients (87.9%) were taking the 1.0 mg dose of semaglutide. When compared with non-SGLT2i-treated patients, no differences in baseline characteristics were found between groups.

Outcomes

During a median follow-up of 6 months, the overall mean serum HbA_{1c} level reduction was 1.3 ± 1.7% with weight loss of 4.7 ± 4.1 kg. The mean percentage weight loss was 5.4% in the study population. Changes in serum HbA_{1c} levels and BW reductions from baseline to 6 months are shown in Figure 2. The proportion of patients who achieved a sustained optimal glycemic control (serum HbA_{1c} level <7.0%) increased from 43.1% to 55.8% (p=0.022) at the last follow-up as shown in Figure 3A. The proportion of

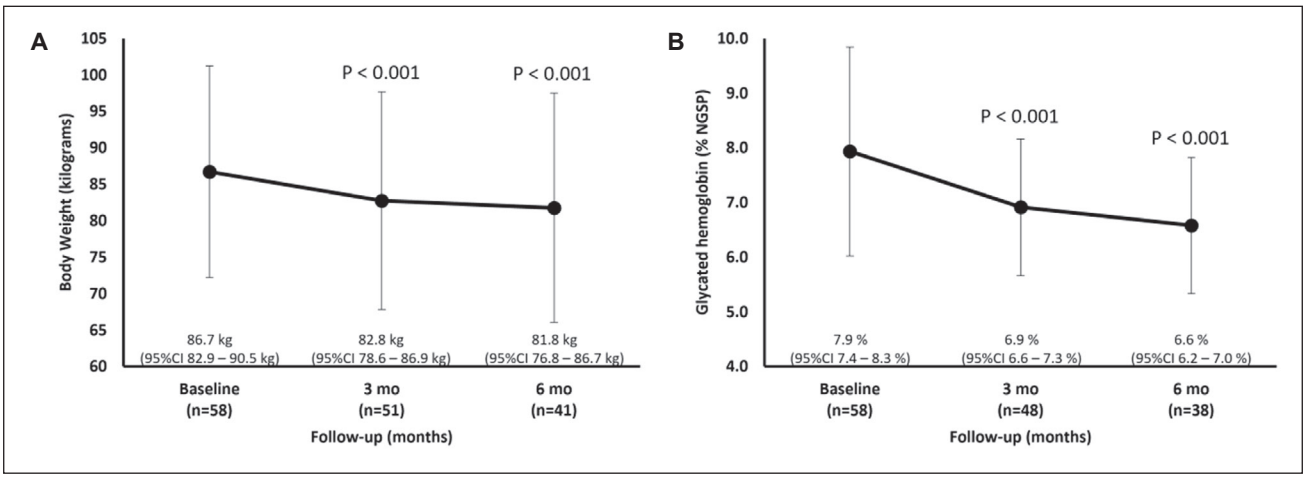

Figure 2. Changes in clinical effectiveness every 3 months after initiation of semaglutide. **(A)** Change in serum HbA_{1c} level from baseline to 6 months. **(B)** BW reduction from baseline to 6 months (Bar graph indicates mean values with standard deviation and numbers in parenthesis indicates 95% Confidence Interval).

Table 1. Baseline demographic and clinical characteristics of studied patients

	Total (N = 58)	SGLT2i-treated group (N = 24)	Non-SGLT2i group (N = 34)	*p*
Current age (years)	55.6 ± 15.9	57.4 ± 12.7	54.4 ± 18.0	0.476
<40 years	10.3	4.2	14.7	
40-64 years	62.1	75.0	52.9	
65-74 years	13.8	12.5	14.7	
≥75 years	13.8	8.3	17.7	
Female (%)	29 (50.0)	13 (54.2)	16 (47.1)	0.594
Duration of DM (years)	10.5 (4.0,20.3)	11.5 (7.0,21.8)	8.0 (2.5,20.3)	0.183
Previous GLP-1 RA (%)	14 (24.1)	7 (29.2)	7 (20.6)	0.452
Established ASCVD (%)	4 (6.9)	3 (12.5)	1 (2.9)	0.157
Diabetic Retinopathy (%)*	13 (27.1)	7 (35.0)	6 (21.4)	0.297
Diabetic Nephropathy (%)	24 (41.4)	12 (50)	12 (35.3)	0.263
Baseline HbA_{1c} (%)	7.9 ± 1.9	7.8 ± 1.5	8.1 ± 2.1	0.570
<7.0%	43.1	37.5	47.1	
7.0-7.9%	12.1	25.0	2.9	
8.0-8.9%	15.5	25.0	8.8	
≥9.0%	29.3	12.5	41.2	
Baseline BW (kgs)	86.7 ± 14.5	85.9 ± 12.8	87.3 ± 15.8	0.716
Baseline BMI (kg/m^2)	31.5 ± 4.4	31.7 ± 4.4	31.4 ± 4.5	0.841
23.0-24.9 kg/m^2	3.4	4.2	2.9	
25.0-29.9 kg/m^2	31.0	29.1	32.4	
30.0-34.9 kg/m^2	46.6	50.0	44.1	
≥35.0 kg/m^2	19.0	16.7	20.6	
Concomitant anti-DM medications (%)				
Sulfonylurea	17.2	20.8	14.7	0.543
Metformin	72.4	79.2	67.6	0.334
Thiazolidinedione	32.8	37.5	29.4	0.518
Insulin	19.0	16.7	20.6	0.708
Duration of usage (months)	6.0 (3.0,12.0)	5.0 (3.0,14.3)	6.0 (3.0,12.0)	0.916
Final dose of semaglutide (%)				
≤0.5 mg per week	12.1	16.7	8.8	0.360
1.0 mg per week	87.9	83.3	91.2	

*Available data = 48 patients

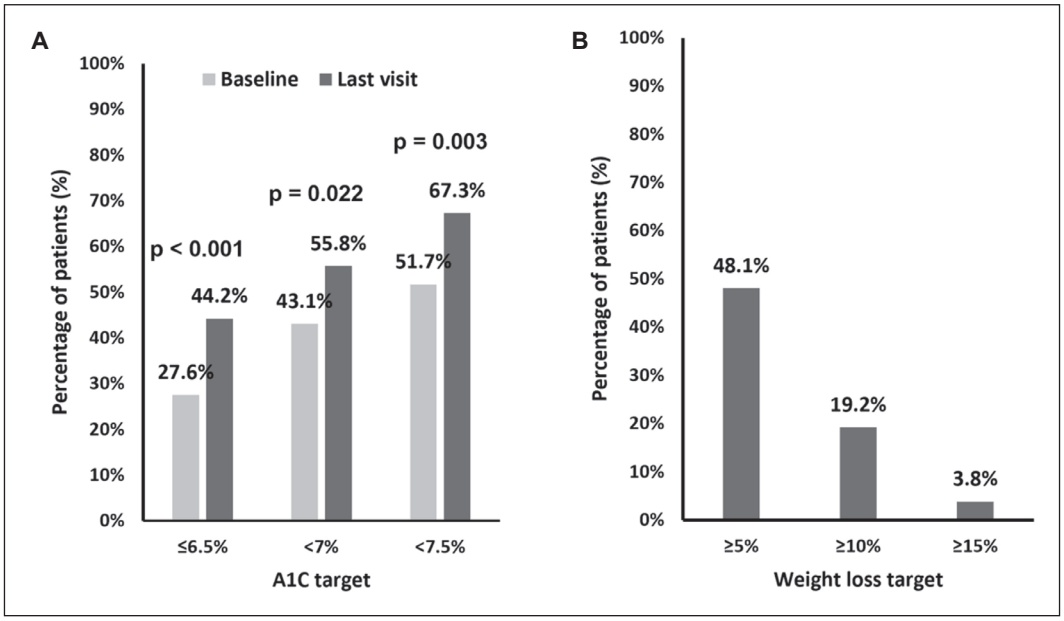

Figure 3. (A) Changes in the proportion of patients who achieved various HbA_{1c} targets from baseline compared to the last follow-up. **(B)** The proportion of patients who achieved various weight loss targets at the last follow-up.

Table 2. Comparisons of results from the present study in Thai patients with observed findings from randomized clinical trials and other published real-world cohorts

Study	Sample size (N)	Mean duration of follow-up (months)	Baseline HbA_{1c} (%)	Baseline BW (kgs)	Mean HbA_{1c} reduction	Mean BW reduction
Present study (Thailand)*	58	6.0	7.9 (7.4, 8.4)	86.7 (82.9, 90.5)	-1.3 (-1.8, -0.9)	-4.7 (-5.7, -3.6)
Pooled analysis of SUSTAIN studies at dose of 1.0 mg[6]	994	8.1	8.2	92.4	-1.6	-6.3
Pooled analysis of SURE studies[12]	1,212	7.7	8.1	101.5	-1.1	-4.7
US Commercially insured and Medicare Advantage patients[13]	1,888	6.6	8.2	N/A	-0.9	N/A
Wales, United Kingdom[14]	189	6.0	9.3	101.8	-1.2	-3.0
Copenhagen, Denmark[15]	119	12	7.7	99.0	-0.8	-3.5
Umbria, Italy[16]	216	12.0	8.4	94.6	-0.8	-3.1
Novara, Italy[17]	258	12.0	8.0	92.5	-1.1	-5.3
Spain[18]	166	24.0	7.5	98.5	-0.9	-9.7

*Parenthesis indicated 95% Confidence Interval

patients who attained sustained weight loss at various targets is shown in Figure 3B. The proportion of patients reaching both HbA_{1c} targets of <7.0% and 5% weight loss was 27.8% at the last follow-up.

Larger reductions in serum HbA_{1c} levels but the least BW reduction were observed within the stratum of patients with baseline serum HbA_{1c} level of >9.0% when compared with those with lower baseline serum HbA_{1c} levels as demonstrated in Figure 4. Serum HbA_{1c} level was reduced from baseline to the last follow-up in all subgroups according to background medication use and there was no discernible pattern in serum HbA_{1c} level reductions in these subgroups as shown in Figure 5.

There were no differences between clinical effectiveness of semaglutide in the SGLT2i-treated and non-SGLT2i treated group.

Among SGLT2i-treated patients, overall mean serum HbA_{1c} level reduction when compared with non SGLT2i-treated patients was 1.1 ± 1.7% and 1.4 ± 0.4%, respectively (p=0.342). Body weight reductions in SGLT2i group: 4.4 ± 3.9 kg and non-SGLT2i group: 4.9 ± 4.2 kg did not show statistical significance (p=0.548). Among patients in the SU subgroup, a larger reduction in serum HbA_{1c} level was observed when compared with patients without SU as a background medication (1.8 ± 1.0% and 1.2 ± 1.8%, respectively (p=0.023). Smaller reductions in BW were observed in patients on background insulin treatment compared with non-insulin subgroups but these did not show statistical significance (2.8 ± 2.3 kg vs 5.1 ± 4.3 kg, p=0.139).

When accounting for previous use of other types of GLP-1 RA, the GLP-1 RA-naive stratum had more BW reduction when compared with the stratum with prior GLP-1 RA use (-5.5 ± 4.3 kg vs -2.0 ± 1.8 kg, p<0.001).

The most common adverse events were GI side effects with mild to moderate severity, occurring primarily during dose escalation. Gastrointestinal side effects included

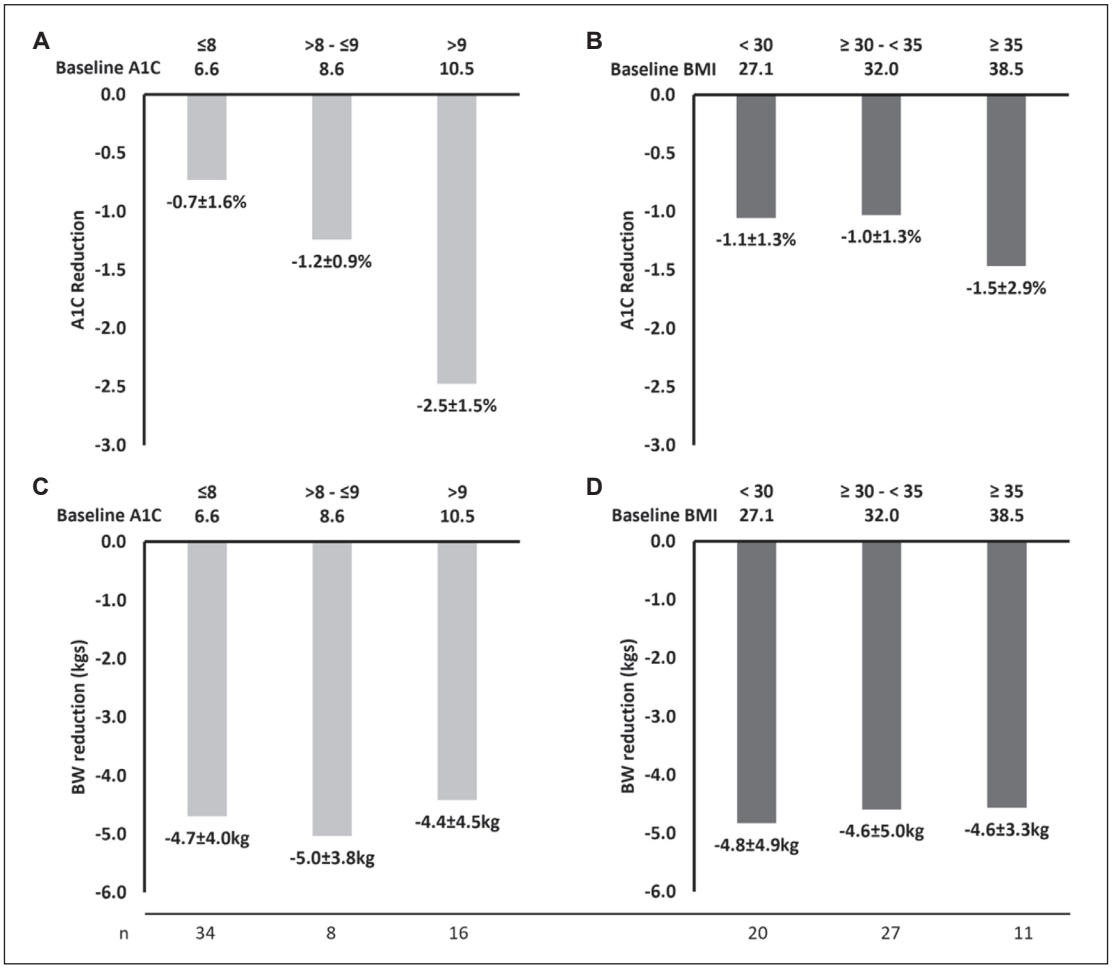

Figure 4. (A) Changes in serum HbA_{1c} level from baseline to the last follow-up according to the stratum of serum HbA_{1c} levels. **(B)** Changes in serum HbA_{1c} level from baseline to the last follow-up according to the stratum of BMI categories. **(C)** Changes in body weight from baseline to the last follow-up according to the stratum of serum HbA_{1c} levels. **(D)** Changes in body weight from baseline to the last follow-up according to the stratum of BMI categories.

nausea and vomiting in 17 patients (29.3%), diarrhea in 3 patients (5.2%), and constipation in 3 patients (5.2%). Treatment was discontinued due to GI side effects only in 3 patients (5.2%) within 3 months after usage. No cases of pancreatitis, cancer, or progressive diabetic retinopathy were observed. No severe hypoglycemia was documented during the study period.

DISCUSSION

In this study, once weekly semaglutide injection was associated with short-term sustained glycemic control and weight loss among persons with T2DM and obesity in Thailand comparable with what has been observed in RCT and other RWE as summarized in Table 2.

Our data suggested that these findings were consistent across subgroups based on background medication despite more frequent use of TZD in our cohort. The result of this study can be used to inform the decisions of healthcare service users to prioritize novel treatments for universal coverage. Although RCTs are important for demonstrating treatment efficacy and safety, it is also important to collect additional evidence in a real-world setting to validate results from the RCT setting because many factors like ethnicity, demography, healthcare infrastructure, service delivery and reimbursement system may affect the outcome. The result of this study represented the RWE of semaglutide in a private setting in Thailand, but the data may be used for policy formation to prioritize novel treatments in resource-limited LIMC's.

In contrast with observed findings in the SURE program,[8-12] there was no clear relationship between the use of background oral medications with the changes in HbA_{1c} or BW reduction except in patients with SU as a background medication. The different findings between the SURE program and our study might be explained by the different nature of a real-world clinical setting and the relatively lower baseline serum HbA_{1c} level and BMI in our study to begin with. The lower baseline serum HbA_{1c} level of our patients (7.9%) when compared with the SUSTAIN program (8.0%-8.4%) and in the SURE studies (8.1%) may have contributed to the comparatively lower reduction in serum HbA_{1c} level from baseline to the last follow-up visit.

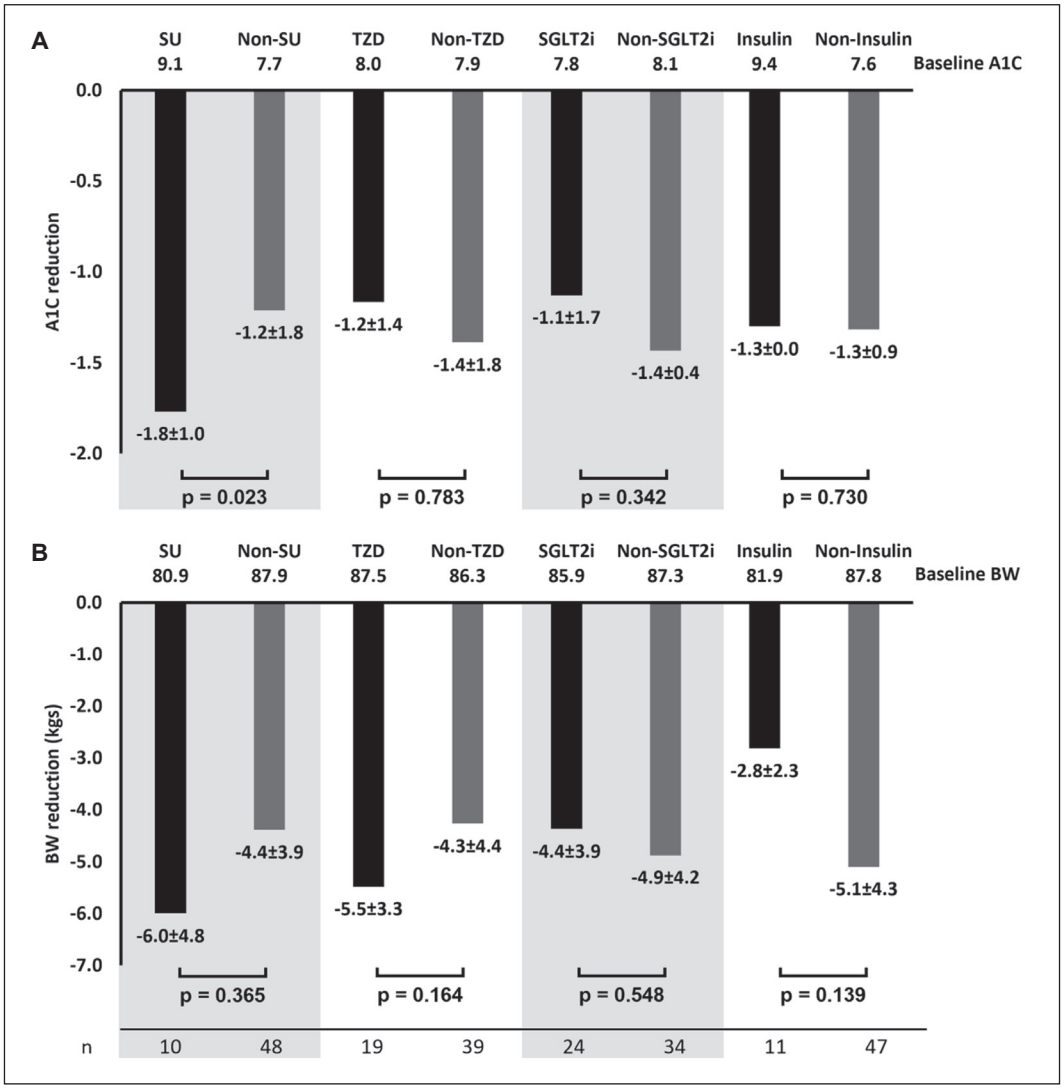

Figure 5. (A) Changes in serum HbA_{1c} level from baseline to the last follow-up according to the background anti-diabetic medication. **(B)** Changes in body weight from baseline to the last follow-up according to the background anti-diabetic medication.

However, the BW reduction in our study (-4.7 kg) was comparable to the SUSTAIN trials (-3.5 kg to -6.4 kg) and the SURE studies (-4.7 kg). However, it should be noted that the small sample size of our SU cohort (only 17.2% of all patients) and relatively high baseline serum HbA_{1c} (9.1%) in this cohort could potentially affect the observed larger serum HbA_{1c} reduction in our study.

It is well-known that the concomitant use of other anti-diabetic drugs with a potential weight gain effect may mask the weight benefit of GLP-1-RA. However, pioglitazone has beneficial effects to treat both hyperglycemia and non-alcoholic fatty liver disease (NAFLD).[22] This inexpensive generic medication has been used frequently in our diabetes center at a lower dose of 15 mg/day. Combination therapy with semaglutide and low-dose pioglitazone might attenuate the risk of weight gain associated with pioglitazone as shown in the present study. Our study validated the effectiveness of and limited side effects associated with low-dose pioglitazone as a background medication in patients who have added once-weekly semaglutide.

Both SGLT2i and GLP-1 RA have well-documented cardiovascular and renal benefits and have potential additive benefits for combination usage.[23-25] However, there is limited data in clinical trials that combined the use of both classes of medication. Based on our study, this combination had generally safe profiles but did not demonstrate superior reductions in serum HbA_{1c} level or BW compared with other background medications. Further studies including cost-effectiveness analysis are required to clarify the role and additive benefits of this combination therapy in people with T2DM as a primary preventive strategy for cardiovascular risk reduction. The proportion of patients discontinuing treatment due to adverse events within one month in our study was 2.0% and lower than in the SURE studies (9.5%) and the SUSTAIN clinical trial program (≤15%).[6-12] This highlighted that semaglutide was very well tolerated in real-world practice among South-east Asian patients.

Long-term adherence to GLP-1 RA treatment may be affected by other issues such as lack of affordability or

injection-related burden rather than GI adverse events. In the retrospective SPARE observational study among patients initiating semaglutide in a specialist endocrinology practice in Canada which was publicly reimbursed, the discontinuation rate was found at 17.3%, which was higher than that reported in the RCT setting but lower when compared to other real-world evaluations of GLP-1 RA medications.[26] The affordability of novel anti-diabetic medications has been a major factor impacting medication adherence in LMICs.[27] Therefore, it is necessary to conduct cost-effectiveness studies that could facilitate wider acceptability and usage among patients in various settings with different healthcare policies.

There were several limitations which could have influenced our results. First, the limitations related to the observational nature of this study and a single data source from a specialized diabetes center in Thailand should be acknowledged. Uncontrolled factors such as changes in lifestyle, concomitant medication use, and verification of medication adherence could confound the observed effectiveness of semaglutide in this study.[28] However, to the best of our knowledge, this study is the first RWE of semaglutide injection among Southeast Asian patients. Our study also provided insight on how semaglutide was initiated and used in routine clinical practice. Second, the small number of study participants and relatively short duration of follow-up in our study may limit the generalizability of the results. Third, this study was not powered for subgroup analyses and only reliably identified potential relationships between baseline variables and treatment effects. It should be interpreted with caution because of the small number of patients in each subgroup. Finally, the present study only assessed the clinical effectiveness of GLP-1 RA in terms of glycemic and BW reductions. The hard endpoints of treatments such as cardiovascular disease, progression to end-stage renal disease, and mortality risk reduction need to be further studied in a multi-center setting with a longer follow-up period.

CONCLUSION

Initiation of once weekly semaglutide injection in this single center study was associated with short-term glycemic control and weight loss among persons with T2DM and obesity in Thailand comparable with what have been observed in randomized clinical trials and other RWE.

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

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

Author Disclosure
The authors declared no conflict of interest.

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ORIGINAL ARTICLE

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Obesity Awareness and Its Relationship to Sociodemographic Characteristics of Filipino Adults: A Survey Among Work-From-Home Employees in Metro Cebu, Philippines*

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Abstract

Objective. Awareness and substantial understanding of obesity are essential components in its prevention and treatment. This study aimed to determine the degree of obesity awareness and its relationship to various sociodemographic characteristics among Filipino adults working from home (WFH).

Methodology. This is a cross-sectional survey conducted in Metro Cebu, Philippines. Included were non-healthcare WFH professionals aged 18-64. Researcher-made Obesity Awareness Questionnaire (OAC-20) was used.

Results. A total of 458 employees participated in the study; mean age was 30.33 years (SD=6.96), mostly female (71.40%) and majority single (77.07%). The mean obesity awareness score was 79.18% (SD=9.02). Age (p=0.198), BMI (p=0.397), work hours/day (p=0.101), and hours of physical activity/day (p=0.458) were not associated with obesity awareness. Similarly, male vs. female (p=0.515), and single vs. married respondents (p=0.629) did not differ significantly in terms of average scores. However, higher educational attainment (p=0.044) and higher socio-economic status (p=0.002) were significantly associated with higher obesity awareness scores.

Conclusion. The surveyed WFH adults were aware of the majority of the important concepts on obesity. Educational attainment and socio-economic status were significant determinants of obesity awareness.

Key words: obesity, awareness, work-from-home

INTRODUCTION

Obesity is an accumulation of excess body fat which if not treated, may lead to other serious debilitating health conditions. Unfortunately, the worldwide prevalence of obesity has tripled between 1975 and 2016. In 2016, the World Health Organization (WHO) estimated that more than 1.9 billion adults were overweight and over 650 million were obese.[1] In the Philippines, the prevalence of obesity shows a gradually increasing trend as well. According to the recent Expanded National Nutrition Survey (2018), the prevalence of obesity increased from 20.2% in 1998 to 37.2 percent in 2018.[2]

The employed sector is not exempt from the obesity pandemic. The 2010 National Health Interview Survey conducted in the United States of America (USA) revealed that those with longer working hours and who work in companies with a large number of employees were mostly overweight or obese.[3] A local study showed a 57% prevalence among employees, most of whom have sedentary work.[4] This problem is expected to worsen with the recent work environment restrictions brought about by the coronavirus pandemic. Due to the work-from-home (WFH) policies intermittently imposed for long periods from 2020-2021, an overall decrease in physical activity and an increase in meal frequency and snacking was noted among the adult working population.[5,6]

Various disease conditions are linked to obesity such as cardiovascular disease, type 2 diabetes mellitus (T2DM) and cancer among others.[1] It is concerning that in the 2020 Philippine Statistics Authority (PSA) data, 5 of the 10 leading causes of death were obesity-related complications.[7]

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Obesity is even a risk factor for the dreaded severe coronavirus (COVID-19) infection which continues to wreak havoc on our country and the world.[8] With its devastating effects on health, WHO declared an obesity epidemic in 1997 and spearheaded public awareness campaigns. They also developed "A Global Action Plan on Physical Activity for 2018-2030," to address this pressing problem.[1]

In our country, the National Nutrition Council of the Department of Health conducted virtual forums on obesity awareness to continue the advocacy, promotion and provision of health and nutrition information to the general public during the COVID-19 pandemic.[9] Moreover, various medical societies formulated clinical practice guidelines to guide clinical practitioners. In the Philippines, there are clinical practice recommendations on obesity from the Philippine Association for the Study of Overweight and Obesity (PASOO) and the Family Medicine Research Group (FMRG) Guidelines of the University of the Philippines-Philippine General Hospital.[10]

Huge advancements in diagnosis have taken place over the years. While body mass index (BMI) is still commonly used to diagnose obesity, numerous imaging procedures are now utilized to refine this diagnosis. Similarly, development of obesity treatment strategies proceeds at an accelerated pace, with numerous pharmacologic and surgical interventions to complement the fundamentally crucial adherence to diet, lifestyle and behavioral modification.[11] Multiple programs from government and the private sector were initiated to help solve the obesity problem, including information campaigns and lay fora on obesity and the operation of weight loss clinics.[12,13]

Unfortunately, in spite of all these interventions, the incidence of obesity continues to rise. Awareness and a substantial understanding of its pathophysiology and its consequences are essential components in its prevention and treatment. Several studies involving students, employees and the general population revealed that these cohorts have ample knowledge on the basic concepts of obesity, specifically its risk factors.[14-16] However, one study highlighted the public's unawareness on the link of obesity to cancer.[17] Majority were also unfamiliar about treatment options and most do not try to prevent themselves from becoming obese.

To date, there is no local data evaluating the degree of knowledge of this disease, especially among the employed, who are at most risk of becoming obese due to their current WFH set up. We lack evidence locally whether sociodemographics play a role in influencing the degree of obesity awareness.

The general objective of this study was to determine the awareness on obesity and its relationship to various sociodemographic variables among Filipino adults WFH in Metro Cebu, Philippines. The specific objectives were: (1) to describe the study population's sociodemographic profile; (2) to assess their level of awareness according to the following: obesity as a disease and its risk factors, complications, diagnosis and management of obesity and (3) to determine if a relationship exists between the degree of obesity awareness and the sociodemographic factors studied.

METHODOLOGY

This was a cross-sectional survey conducted in Metro Cebu, Philippines, which included Filipino adult WFH employees aged 18 years and above. Subjects were non-healthcare professionals and were self-employed or employed in a company under a full WFH scheme/set-up or combined office work-WFH set-up during the survey period.

Excluded from the study were healthcare professionals such as medical doctors, nursing professionals, midwives, dentists, pharmacists, nurse practitioners, physician's assistants, emergency medical technicians, dieticians and nutritionists, and physiotherapists.[18]

A sample size of at least 384 respondents was computed using EPI Info Stat Calculator for population surveys/descriptive studies and was based on the Philippines Statistics Authority (PSA) - Region VII's labor force census.[19]

This study sampled workers using the non-probability snowball sampling method from specified cities in Metro Cebu to ensure that these areas are well represented. Researchers had key contact persons—WFH employees from Cebu City, Mandaue City, Lapu-Lapu City and Talisay City. These key persons were asked to recruit other participants to join this study. Recruitment was done via various social media apps and email until the required sample size was surpassed.

The respondents were asked to answer a researcher-made instrument. The first part of the tool gathered various sociodemographic characteristics of WFH employees such as age, sex, marital status, height, weight, BMI, highest educational attainment, socio-economic status based on monthly household income, hours of work per day and hours of physical activity/exercise per day.

The second part, the Obesity Awareness Questionnaire (OAC-20), assessed the respondents' awareness on obesity. OAQ-20 is made up of 20 items covering the following: obesity as a disease and its risk factors (7 items), complications of obesity (7 items), diagnosis of obesity (2 items) and management of obesity (6 items). All statements in the questionnaire were true regarding obesity. Respondents answered questions covering the aforementioned areas using a 5-point Likert Scale: 5-strongly agree, 4-agree, 3-undecided, 2-disagree and 1-strongly disagree.

The research instrument was subjected to content validity and reliability testing procedures. Content validity was done by a panel of experts: endocrinologists, clinical nutritionists and other internists. Content Validity Indices such

as Item-level Content Validity Index (I-CVI) and Scale-Level Content Validity Index (S-CVI) were calculated. Furthermore, Attribute Agreement Analysis, a form of inter-reliability measure, was done to assess whether the experts' relevance ratings were consistent with one another and if observer agreement was due to chance or not.

Content validation was done in the original 22-item tool. I-CVI is 1.00 for all items except 8 and 18. S-CVI is 0.98. All content validity indices were acceptable, thus, the proposed obesity awareness questionnaire achieved a satisfactory level of content validity. Items 8 and 18 were omitted in the final tool. To determine whether agreement among experts was due to chance, the p-values for each attribute's Fleiss Kappa were compared to the significance level. With the $p<0.05$, all appraiser agreements then were not due to chance. Assessments made for each item, therefore, were consistent among appraisers and thus, reliable.

Finally, a total of 3 rounds of pre-testing was conducted. Results of these pre-tests were the bases for internal consistency or reliability testing (Cronbach's alpha), and item analyses. The tool was evaluated as a unidimensional instrument, all 20 items measuring a single latent trait, in this case, obesity awareness. The over-all Cronbach alpha was 0.86. With a relatively high coefficient, this means that the items of the survey can reliably assess the same construct, obesity awareness, whether among healthcare professional or lay persons.

The Cronbach alpha for each sub-construct were as follows: obesity as a disease and its risk factors (7 items), 0.7; complications of obesity (7 items), 0.9; diagnosis of obesity (2 items), 0.4; and management of obesity (4 items), 0.4. The low value of alpha for the last two groupings could be due to the small number of questions, which came from a decision to include only extremely necessary items.

For example, the clinical diagnosis of obesity relies primarily on the patient's BMI and/or waist circumference, hence, only these 2 were included in the assessment of awareness on the diagnosis of obesity.

Furthermore, the proposed instrument measures obesity awareness among the general public who we do not expect to be aware of less commonly used diagnostic measures which are costly, unstandardized or not widely available. So, BMI and/or waist circumference would suffice in both clinical and general settings. The same principle was applied in the finalization of the items for the management of obesity.

Nevertheless, we feel that these low ratings in the last 2 groupings should not devalue the proposed instrument, as alphas have been proven to be affected by the number of questions. Also, all 4 were necessary sub-constructs of obesity awareness as per content validation. There is a need to look into all these key areas to properly represent the entire spectrum of obesity awareness.

Remarkably, the Cronbach alpha for the non-healthcare professionals pre-testing and that of the healthcare professionals pre-testing were similar, 0.79 and 0.80, respectively. With similar Cronbach alpha values ($\cong$0.80) for both test groups, this further indicates that items meant to assess awareness obesity may work for both populations.

The validated questionnaire was also translated into the *Bisaya* language. Forward translation was done by a certified linguist who is fluent in both the source and target language and is knowledgeable about health care terminology. The linguist was briefed by the authors regarding the content area of the construct of the instrument in the desired target language. Another linguist of the same caliber was tasked to perform blind back-translation.

The instructions, items and the response format of the translated and back-translated versions of the instruments were compared to the original regarding ambiguities and discrepancies of words, sentences and meanings. All comparisons were discussed, ambiguities and discrepancies were dealt with and resolved. Consensus was achieved thus generating the Bisaya version of the Obesity Awareness Questionnaire (OAC-20-B). The said version was subjected to the same rounds of pre-testing as indicated above. The over-all computed Cronbach's alpha was 0.90.

Actual data collection was done from June 15 to July 31, 2021. Due to COVID-19 pandemic restrictions and for the safety of researchers and respondents, a self-administered survey was conducted online via Google Forms. The study was approved by the Cebu Doctors' University Hospital Technical Review Board and Institutional Research Ethics Committee.

In the analysis of data, descriptive statistics (mean, SD, median, IQR, minimum and maximum Values) were used to describe the distribution of WFH employees in terms of numerical profile characteristics and obesity awareness assessment scores of respondents. Frequency and simple percentage were used to determine the distribution of respondents in terms of different categorical variables. Spearman's Correlation was used to determine if there was association between numerical variables such as awareness scores and numerical/ordinal profile variables. Mann-Whitney U Test was used to determine if there was significant difference between 2 groups of respondents in terms of their obesity awareness scores. For all tests, confidence interval was set at 95%, relationship or comparison significant at <0.05, all hypotheses were tested at 0.05 level of significance. Data were entered with Microsoft Excel Spreadsheet and then analyzed with Minitab version 19.0 for Mac Mojave OS.

RESULTS

A total of 458 WFH employees participated in the study. As shown in Table 1, the mean age of the cohort was 30.33 years and ranged from 18-64 years. There were 327 females

(71.40%) and 131 males (28.6%). The married respondents comprised 22.93% of the population and 77.07% were single.

Self-reported height ranged from 1.25-1.88 meters while the self-reported weight ranged from 30-155 kg. Majority of the respondents had reported BMIs that were above normal. Using the Asia-Pacific cutoff, the computed BMI revealed 20 (4.42%) respondents were underweight, 159 (35.18%) were normal, 85 (18.81%) were overweight, 148 (32.74%) were pre-obese and 40 (8.85%) were obese.

Table 1. Sociodemographic characteristics of work-from-home employees in Metro Cebu

Respondents' sociodemographic profile	N=458		
	Mean (SD)	Median (IQR)	Min-Max
Age in years	30.33 (6.96)	29 (26-33)	18-64
Height in m	1.59 (0.09)	1.58 (1.52-1.65)	1.25-1.88
Weight in kg	63 (15.73)	60 (53-70)	30-155
BMI in kg/m^2	24.75 (5.13)	24.1 (21.7-26.6)	12.82-64.08
	Number	%	
Age groups			
Teens	2	0.44	
Twenties	250	54.59	
Thirties	168	36.68	
Forties	27	5.90	
Fifties	8	1.75	
Sixties	3	0.66	
BMI classification (Asian)			
Underweight	20	4.42	
Normal	159	35.18	
Overweight	85	18.81	
Pre-Obese	148	32.74	
Obese	40	8.85	
Sex			
Female	327	71.40	
Male	131	28.60	
Marital status			
Single	353	77.07	
Married	105	22.93	
Highest educational attainment			
Elementary	2	0.44	
High school	16	3.49	
College	378	82.53	
Graduate (master's) studies	59	12.88	
Post-graduate (doctorate) studies	3	0.66	
Socio-economic status based on monthly household income			
Level 1-Less than PhP 10,481	32	7.06	
Level 2-Between PhP 10,481 and PhP 20,962	127	28.04	
Level 3-Between PhP 20,962 and PhP 41,924	164	36.20	
Level 4-Between PhP 41,924 and PhP 73,367	63	13.91	
Level 5-Between PhP 73,367 and PhP 125,772	33	7.28	
Level 6-Between PhP 125,772 and PhP 209,620	16	3.53	
Level 7-PhP 209,620 and above	18	3.97	
Hours of work per day			
Less than 8 hours	93	20.31	
8 hours	314	68.56	
12 hours	39	8.52	
More than 12 hours	12	2.62	
Hours of physical activity/exercise per day			
Less than 30 minutes	237	51.97	
About 30 minutes	113	24.78	
About 1 hour	72	15.79	
About 2 hours	21	4.61	
More than 2 hours	13	2.85	

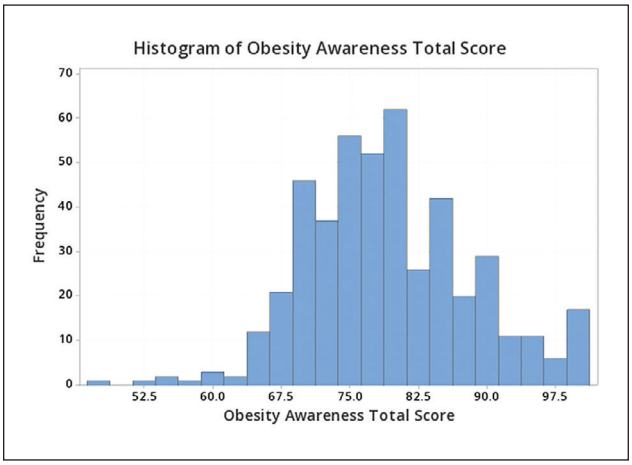
Majority of the respondents were college graduates (82.53%) and more than 50% of respondents had level 2-3 monthly household income (PhP 10,481 to 41,924). Sixty-nine percent of the WFH employees worked 8 hours per day. It is noteworthy that more than half of the respondents had only 30 minutes or less of physical activity/exercise per day.

As shown in Figure 1, the mean total obesity awareness score of the respondents was 79.18 with a standard deviation of 9.02 points. The lowest score was 47. About 25% of the awareness scores in the sampled population were less than or equal to 73 (Q1). The median score was 78, which means that half of the sampled population scored below this value. About 25% of the scores in the sampled population were greater than 84, i.e., 75% of the respondents scored 84 and below in obesity awareness. The highest obesity awareness score was 100.

To better understand the respondents' awareness on obesity, item scores were computed and these are presented in Table 2. When it comes to obesity as a disease and its risk factors, most respondents were aware that hormonal imbalances can affect a person's weight. The mean score for this item was 4.30 (SD=0.63). WFH employees, however, were least aware of how common obesity is among Filipinos. The mean score for that item was 3.51 (SD=1.23).

In terms of complications, most respondents were aware that obesity is associated with elevated blood pressure and cardiovascular diseases as evidenced by a mean item score of 4.22 (SD=0.78). However, they were relatively less aware that obesity is actually associated with T2D, infertility, colon cancer and severe COVID-19 infection.

As far as diagnosis of obesity is concerned, most of the respondents were aware that BMI is commonly used to



Mean	SD	Minimum	Q1	Median	Q3	Maximum
79.18	9.02	47.00	73.00	78.00	84.00	100.00

Figure 1. Summary of respondents' total obesity awareness scores.

Table 2. Respondents' scores on obesity awareness

Subscales and items on obesity awareness	N=458			
	Mean (SD)	Median (Q1-Q3)	Subscale awareness ranking	Overall awareness ranking
Obesity as a disease and its risk factors				
1-Obesity is a disease.	3.96 (1.06)	4 (3-5)	5	12
2-Obesity is not rare among Filipinos.	3.51 (1.23)	4 (2-4)	7	19
3-Overeating is not the only known cause of obesity.	4.17 (0.87)	4 (4-5)	3	7
4-People who have minimal physical activity are at risk for obesity.	4.19 (0.86)	4 (4-5)	2	6
5-Certain medications such as steroids may contribute to weight gain.	3.90 (0.83)	4 (3-5)	6	13
6-Genes have a role in the development of obesity	4.05 (0.74)	4 (4-5)	4	10
7-Hormonal imbalances can affect a person's weight.	4.30 (0.63)	4 (4-5)	1	2
Complications of obesity				
8-Obesity is associated with elevated blood pressure and cardiovascular diseases.	4.22 (0.78)	4 (4-5)	1	4
9-Being overweight increases one's risk of developing colon cancer.	3.72 (0.83)	4 (3-4)	6	16
10-Obesity is a risk factor for severe COVID 19 disease.	3.68 (1.02)	4 (3-5)	7	17
11-Type II Diabetes mellitus is related to obesity.	3.79 (0.85)	4 (3-4)	4	14
12-Overweight can be related to infertility.	3.73 (0.91)	4 (3-4)	5	15
13-Obesity has been linked to Fatty Liver and Gallbladder diseases.	4.08 (0.79)	4 (4-5)	2	9
14-Depression is considered both a risk factor and a complication of obesity	4.04 (0.81)	4 (4-5)	3	11
Diagnosis of obesity				
15-Body Mass Index is used to determine whether a person is overweight or not.	4.31 (0.74)	4 (4-5)	1	1
16-Obesity can be diagnosed by determining Waist Circumference.	3.32 (1.09)	3 (2-4)	2	20
Management of obesity				
17-When one is trying to lose weight, it is ideal to seek professional advice.	4.19 (0.76)	4 (4-5)	2	5
18-Moderate intensity exercises, such as brisk walking, can help you achieve a healthy weight.	4.22 (0.71)	4 (4-5)	1	3
19-Taking weight loss medications is not enough to manage obesity.	4.12 (0.87)	4 (4-5)	3	8
20-There are surgical options that can help treat obesity.	3.68 (0.87)	4 (3-4)	4	18

Table 3. Awareness on obesity and sociodemographic characteristics

Variables	Computed Values	*p*
Obesity awareness and		
Age	0.060 [a]	0.198
Sex	Female η_1=78; Male η_2=78[b]	0.515
Marital status	Married η_1=78; Single η_2=79[b]	0.629
Body Mass Index	0.040[a]	0.397
Highest educational attainment	0.094[a]	0.044
Socio-economic status	0.147[a]	0.002
Hours of work per day	0.077[a]	0.101
Hours of physical activity per day	0.035[a]	0.458

[a]Spearman's rho; value computed using Spearman's Rank Correlation; significant at <0.05
[b]Median values; Comparison done with Mann-Whitney U Test; significant at <0.05

determine if a person is overweight or not, as evidenced by a mean item score of 4.31 (SD=0.74). However, much of the sampled population did not know that obesity can also be diagnosed by measuring waist circumference as well. A mean score of 3.32 (SD=1.09) supported this finding.

Respondents were also assessed in terms of their awareness in the management of obesity. WFH employees were aware that moderate intensity exercises can help achieve a healthy weight (mean score 4.22, SD=0.71) and that professional advice should be sought before losing weight (mean score 4.19, SD=0.76). On the other hand, only a few were aware that there are surgical treatment options for obesity (mean score 3.68, SD=0.87).

Table 3 presents the results when assessing the relationship (or differences in terms sex and marital status) between the obesity awareness scores and various sociodemographic variables of the respondents. Age (p=0.198), BMI (p=0.397), hours of work per day (p=0.465) and hours of physical activity per day (p=0.765) were not associated with obesity awareness among the studied population. Sex (p=0.515) and marital status (p=0.629) were also not significantly associated with obesity awareness. On the other hand, educational attainment (p=0.044) and socio-economic status (p=0.002) were both associated with the respondents' obesity awareness scores. For highest educational attainment and obesity awareness, the computed value (Spearman's Rho) of 0.1 indicates that a positive monotonic correlation exists between the 2 variables. This means that higher levels of education were associated with higher obesity awareness scores. There was also a positive monotonic correlation between socio-economic status and obesity awareness scores as indicated by the computed value of 0.147. Similarly, higher socio-economic statuses were associated with higher obesity awareness scores. It must be noted, however, that these correlations do not imply a causal relationship (cause and effect).

DISCUSSION

In the Philippines, the prevalence of obesity showed a gradual increasing trend with 3 out of 10 adult Filipinos being overweight and obese.[2] To date, there are no studies focusing on obesity awareness among Filipinos, much less on the locally-employed sector.

The sampled population of Filipino adults WFH in Metro Cebu were aware of most of the important concepts on obesity as a disease and its risk factors, complications, diagnosis and management as evidenced by an overall average score of 79.18%, covering therefore nearly 80% of the salient facts regarding obesity. Perhaps the ease of accessing information through the internet may be the reason for their high level of awareness. The results in other countries are polarized with studies in China, Pakistan and India revealing cohorts who were knowledgeable on the core principles of obesity while studies done in the USA and United Kingdom (UK) showed otherwise.[14-17,20]

The item that the respondents were most aware of is that BMI is a diagnostic tool for obesity. This is because BMI is commonly used and is regarded as the most useful population-level measure of overweight and obesity.[1] The second highest ranking in the overall awareness is that hormonal imbalances can affect a person's weight. Hormonal influence is usually cited as one of the causes of obesity.[21] Although we are unsure how the respondents are particularly aware of this fact, it is nevertheless reassuring to know that this cohort realizes that obesity is a multi-factorial condition, requiring holistic treatment and inputs from multiple disciplines.

On the other hand, the knowledge of waist circumference as an obesity diagnostic tool had the lowest awareness score. This was similarly noted in a study by Dunkley et al., (2009) were almost half the subjects had no previous knowledge of the importance of waist circumference measurement in screening for obesity.[22]

Majority of the respondents are not aware of the increasing prevalence of obesity in the country. This might be because many people are unable to recognize those who are already overweight/obese which is supported in studies by Nanda (2021) and DeVille-Almond et al (2011).[20,23] This may be problem because recognizing a disease condition precedes treatment. In spite of the development of obesity treatment strategies,[11] our study revealed that too many respondents were still unaware that there are surgical options for treating obesity. This is likely because surgery is not usually discussed with the patient at the onset of treatment. Lifestyle and behavioral modifications are rightfully given greater emphasis in obesity management.[1] Surgical options such as gastric bypass are only suitable for a select group of morbidly obese patients or obese patients with co-morbidities.[11]

Only a few respondents recognized cancer as an obesity-related complication,[17] including in our study were mostly it ranked at the bottom half of the overall awareness ranking. This relatively low awareness regarding obesity and its complications is concerning since 5 of the 10 leading causes of death are obesity-related according to the 2020 PSA data.[7]

Despite having sufficient awareness about obesity, the majority of the respondents had reported BMIs above normal. This is probably due to the significant reduction in physical activity, along with the tendency to overeat due to the sudden shift to WFH set-up for millions of employees around the world, the Philippines included, during the COVID-19 pandemic.[24-27] Another possible explanation of abnormal BMIs despite sufficient awareness is their misperception of weight and obesity, common among overweight/obese adults.[23] Nanda et al., showed that only a few persons knew their BMIs.[20]

In addition, our study showed the respondents were knowledgeable on the nature of obesity, how it develops, what its predisposing factors are, and how to combat the disease. However, they were relatively less informed on its complications. This might be why this cohort is well-versed on obesity but are themselves above their ideal weight. Knowledge can strengthen one's determination to adhere to approved medical measures to treat obesity. By educating the public on the serious complications of obesity, the problem will transcend mere aesthetics and may motivate people to avoid and prevent this medical condition.

A previous study by the Canadian Institute for Health Information (2011) has concluded that there were associations between the sociodemographic factors and the likelihood of being overweight or obese.[28] However, this study did not assess the level of obesity awareness.

Our study also showed that obesity awareness is significantly related to the socioeconomic status. The higher the household monthly income, the higher the level of awareness. This may be due to the more ready access to obesity education similar to a study in Latin America.[29]

There was also a significant relationship between higher educational attainment and obesity awareness. Those with lower educational levels were expected to score low, thus, less likely to be knowledgeable in lifestyle diseases such as obesity.[30] Having said that, it is interesting to note that the latest National Nutrition Survey showed that higher educational attainment had higher odds of becoming overweight/obese.[31] This implies that there is a gap in the knowledge on obesity and in the application of its concepts.

In interpreting the results of the study, there were few limitations that should be considered. Our study was sampled via non-probability, snow-ball sampling due to COVID-19 restrictions. Further, the weight and the height of the respondents were self-reported. Thus, responses were limited to the honesty of the participants. Also, this study did not look into confounding variables such as the participant's access to social media, awareness of current events, current medical conditions and access to a healthcare practitioner which might have influenced awareness.

CONCLUSION

The sampled population of Filipino adults WFH in Metro Cebu were aware of majority of the important concepts on obesity as a disease and its risk factors, complications, diagnosis and management.

Age, BMI, hours of work per day, and hours of physical activity per day were not associated with obesity awareness among the studied population. Similarly, male and female, as well as single and married respondents do not differ significantly in terms of their average obesity awareness scores. However, higher educational attainment and socio-economic status were associated with higher obesity awareness.

Recommendations

This study provides novel information on the awareness on obesity among Cebuanos. However, the researchers recommend that a larger and a more diverse population be studied. Larger studies may also be done to establish cut-off scores. We also recommend community-wide workshops, not just lectures, with greater emphasis on the application of learned concepts.

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

CRediT Author Statement
JLG: Conceptualization, Methodology, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Funding Acquisition; **AC:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Funding Acquisition; **TL:** Conceptualization, Methodology, Investigation, Writing – original draft preparation, Writing – review and editing, Supervision. Project Administration; **AP:** Methodology, Software, Validation, Formal analysis, Writing – original draft preparation, Writing – review and editing.

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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 Malaysia: Results from a Prospective, Non-interventional Real-World Study

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Abstract

Objectives. Insulin degludec (IDeg)/insulin aspart (IAsp; IDegAsp) is a co-formulation of 70% IDeg and 30% IAsp. According to several randomized controlled trials, IDegAsp is effective and safe for patients with type 2 diabetes mellitus (T2DM). A subgroup analysis of the ARISE study was conducted to explore the safety and efficacy of IDegAsp among Malaysian patients with T2DM in real-world settings.

Methodology. ARISE, an open-label, multicenter, non-interventional, prospective study was conducted between August 2019 and December 2020. Adult Malaysian patients with T2DM who were enrolled from 14 sites received IDegAsp as per the local label for 26 weeks. The primary endpoint was change in glycated hemoglobin (HbA1c) levels from baseline to end of study (EOS).

Results. Of the 182 patients included in the full analysis set, 159 (87.4%) completed the study. From baseline to EOS, HbA1c (estimated difference [ED]: –1.3% [95% CI: –1.61 to –0.90]) and fasting plasma glucose levels (ED: –1.8 mmol/L [95% CI: –2.49 to –1.13]) were significantly reduced (p<0.0001). The patient-reported reduced hypoglycemic episodes (overall and nocturnal) during treatment. Overall, 37 adverse events were observed in 23 (12.6%) patients.

Conclusion. Switching or initiating IDegAsp treatment resulted in significant improvements in glycemic control and a reduction in hypoglycemic episodes.

Key words: type 2 diabetes mellitus, insulin degludec, insulin aspart, Malaysia, hypoglycemia

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INTRODUCTION

There has been a steady increase in the global prevalence of diabetes in the last few years. In 2021, over 537 million (1 in 10) adults aged 20 to 79 years had diabetes, while 541 million adults were at a high risk of developing type 2 diabetes mellitus (T2DM).[1] According to the National Health and Morbidity Survey, the overall prevalence of T2DM in Malaysia is estimated to be 18.3%, with roughly one in five adults having T2DM.[2] However, diabetes management is suboptimal in Malaysia.[3]

The Malaysian Clinical Practice Guidelines (CPGs) for the "Management of Type 2 Diabetes Mellitus," recommends the use of glucose-lowering drugs (GLDs; oral or injectable) as monotherapy or in combination, along with lifestyle modifications for the management of T2DM patients with glycated hemoglobin (HbA1c) ≥6.5% or fasting plasma glucose (FPG) ≥6.0 mmol/L.[4] Further, in patients with inadequate glycemic control on maximum doses of oral GLD (OGLD) ± glucagon-like peptide receptor agonist (GLP-1RA), CPGs recommend initiation of once-daily (OD) co-formulation (insulin degludec/insulin aspart; IDegAsp), basal insulin or premixed insulin.[4] The prolonged use of insulin may have associated challenges, including adverse effects such as hypoglycemia and the inconvenience associated with multiple daily injections. Therefore, many patients consider insulin therapy as burdensome and eventually become non-compliant to treatment.[5] To overcome some of these barriers, it is essential to develop a convenient and effective insulin therapy for patients with T2DM.

IDegAsp (Ryzodeg®, Novo Nordisk A/S, Søborg, Denmark), a soluble co-formulation of 70% insulin degludec (IDeg) and 30% insulin aspart (IAsp) is easy to use with its convenient once daily dosing.[6,7] IDegAsp became available in Malaysia in 2018. Effective glycemic control of IDegAsp is due to its unique pharmacodynamic profile. It provides stable basal insulin coverage for 24 hours by the ultra-long-acting IDeg and postprandial control by rapid-acting IAsp. [8] Treatment with IDegAsp is convenient as it requires minimal injections without resuspension and facilitates accurate dosing.[9] Multiple randomized controlled trials (RCTs) have shown the efficacy and safety of IDegAsp,[6,10] as seen in the BOOST clinical trial program.[11-14] A post hoc pooled investigation of five phase 3, open-label, treat-to-target, 26-week RCTs comparing twice-daily (BID) IDegAsp with premixed insulin BID regimen or IDeg OD +IAsp confirmed the safety and efficacy of IDegAsp in a broad patient population with varying characteristics.[15]

However, real-world evidence on the use of IDegAsp is limited. ARISE (A Ryzodeg Initiation and Switch Effectiveness) was an open-label, prospective, single-arm, non-interventional study for 26 weeks, which assessed glycemic control along with other clinical parameters related to the use of IDegAsp in patients with T2DM. This study included patients in Malaysia, South Africa, India, Saudi Arabia, Australia and the Philippines who were either initiated or switched to IDegAsp.[9] We present the results from the Malaysian cohort from a subgroup analysis of the ARISE study.

METHODOLOGY

Study design

The detailed study design has already been published.[9] Briefly, it was an open-label, 26-week multicenter, prospective, non-interventional study conducted from August 2019 through December 2020 in patients with T2DM. Data were collected from 14 sites in Malaysia. The physician decided which patients would be initiated or switched to IDegAsp. Follow-up was for 26 to 36 weeks.

The decision to initiate or switch to IDegAsp was made before baseline and was not dependent on patient inclusion criteria of the current study. Physicians prescribed the initial dose, dose adjustments, dosing frequency of IDegAsp, and discontinued other GLDs. No additional clinical procedures other than the local standard clinical practices were performed.

The study was conducted in accordance with the Declaration of Helsinki. The ethics committee/institutional review board approved the study protocol and patient consent forms for all the sites in Malaysia. The patients were asked to submit written informed consent before study participation. This study is registered in ClinicalTrials.gov (NCT04042441).

Study population

Patients with T2DM (≥18 years of age) who had received anti-diabetic medications other than IDegAsp for at least 26 weeks with an available HbA1c value measured ≤12 weeks before enrolment were included in the study.

Exclusion criteria were patients with mental incapacity, unwillingness to participate, and those who were already on IDegAsp treatment.

Data collection

Data were collected at baseline (visit 1; week 0), at multiple intermediate visits based on the local clinical practice (visit 2×; week 1–25), and at the end of study (EOS) / treatment discontinuation visit (visit 3; the first visit within week 26–36 / at the time of discontinuation).

Study endpoints

The primary endpoint of the study was the change in HbA1c from baseline to EOS. The secondary endpoints were the proportion of patients attaining HbA1c <7.0% at EOS, change in FPG, insulin dose (total, prandial and basal), and body weight from baseline to EOS.

The other endpoints were as follows: patient-reported non-severe hypoglycemic episodes (overall and nocturnal) within four weeks before IDegAsp initiation and within four weeks before EOS, and severe hypoglycemic episodes occurring in the 26 weeks before IDegAsp initiation and during the 26-week study period. Non-severe hypoglycemia was defined as low blood glucose levels at ≤3.9 mmol/L, with or without symptoms, that were managed by the patient without assistance. On the other hand, severe hypoglycemia was a hypoglycemic episode that required assistance from another person to relieve neurocognitive symptoms such as administering carbohydrates or glucagon. A nocturnal event was a hypoglycemic event that occurred during the night. Data on the reasons for starting baseline IDegAsp, the proportion of patients who discontinued treatment during the study period and their reasons for discontinuation were also collected.

Exploratory endpoints included healthcare resource utilization (HRU) in managing diabetes and its complications within 12 weeks before IDegAsp initiation and 12 weeks before EOS or discontinuation.

Statistical analysis

All the patients who signed the informed consent form and initiated IDegAsp treatment were included in the full analysis set (FAS). An enrolment of 1112 patients overall, with a minimum of at least 139 patients in each country, was planned. Statistical basis for determining the number of enrolled patients for the ARISE study has been described previously.[9] The primary endpoint was analyzed via adjusted mixed models for repeated measurements (MMRM). This analysis was conducted using an 'in-study' observation period that included all patients in the FAS with at least one post-baseline HbA1c measurement regardless whether they discontinued IDegAsp or not. The covariates of the adjusted model were baseline HbA1c, HbA1c assessment time, body mass index (BMI), sex, age, study site, and previous GLDs. According to the 'on-treatment' observation period, secondary analyses of the primary endpoint were done. Repeated primary and secondary analyses were conducted to detect the change in FPG, insulin dose and body weight from baseline to EOS and the baseline values of the relevant endpoints were considered as covariates. The primary analysis was conducted via the adjusted MMRM for the in-study observation period for all endpoints, except HRU. The secondary analysis of HRU was conducted using an on-treatment observation period. The on-treatment observation period was a part of the in-study observation period; during this period the patients received IDegAsp and the values measured following the discontinuation of treatment were ignored. Statistical analysis was performed using SAS software version 9.4.

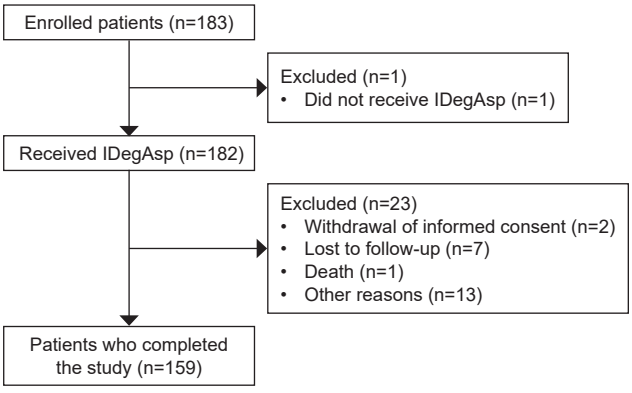

Figure 1. Patient flow through the trial.

RESULTS

Patient demographics and clinical characteristics

Among 1112 eligible patients enrolled in the ARISE study, 205 patients from Malaysia were included. Among them, 187 signed the informed consent, however, only 183 participants attended visit 1. Only 182 patients were initiated or switched to IDegAsp and were included in the FAS (Figure 1). However, only 159 participants (87.4%) completed the study. Nineteen (10.4%) patients discontinued treatment and their reasons for doing so are listed in Supplementary Table 1.

Table 1 represents the baseline demographics and clinical characteristics of the patients. The study enrolled an equal proportion of males and females with a mean age of 56.4 years (standard deviation [SD] 11.88 years). Similar to the population in the global cohort, the Malaysian cohort enrolled those with long-standing diabetes with a mean of 11.2 years (SD 7.99 years), mean BMI of 27.4 kg/m^2 (SD

Table 1. Demographic and clinical characteristics at baseline of patients in Malaysia and for the overall study population (six countries)

	Malaysia N=182[a]	Overall study N=1102[a]
Age, mean (SD)	56.4 (11.88)	58.6 (12.23)
Male, n (%)	95 (52.2)	591 (53.6)
Duration of diabetes (years), mean (SD)	11.2 (7.99)	13.3 (8.33)
Body weight (kg), mean (SD)	71.8 (14.38)	79.5 (19.56)
BMI (kg/m$_2$), mean (SD)	27.4 (4.62)	29.2 (5.86)
HbA1c (%), mean (SD)	10.0 (2.14)	9.8 (1.99)
FPG (mmol/L), mean (SD)	11.0 (4.39)	11.0 (4.22)
Anti-diabetic treatment, n (%)		
OADs only	52 (31.5)	371 (35.1)
Premixed insulin ± bolus insulin (± OADs)	36 (21.8)	232 (21.8)
Basal insulin only (± OADs)	38 (23.0)	230 (21.8)
Basal–bolus insulin (± OADs)	24 (14.5)	137 (13.0)
GLP-RA ± insulin (± OADs)	15 (9.1)	87 (8.2)
Dose of previous prandial insulin (U), mean (SD)	27.0 (22.05)	25.8 (22.84)
Diabetes complications, n (%)		
Diabetic neuropathy	46 (28.8)	216 (24.7)
Diabetic nephropathy	64 (40.0)	178 (20.3)
Cardiovascular disease	27 (16.9)	150 (17.1)
Diabetic retinopathy	20 (12.5)	102 (11.6)
Peripheral vascular disease	1 (0.6)	15 (1.7)

[a] Note that the number of patients differed for the different items.
BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; N, number of patients in the full analysis set; n, number of patients in the subcategory; OAD, oral antidiabetic drug; SD, standard deviation; U, unit.

Table 2. Physicians' reasons for initiating or switching to IDegAsp

	Malaysia N=182	Overall study N=1102
To improve the patient's glycemic control	169 (92.9)	1026 (93.1)
To lower the risk of hypoglycemia	37 (20.3)	291 (26.4)
Flexibility in the dosing regimen	58 (31.9)	286 (26.0)
Fewer injections than basal and bolus therapy	43 (23.6)	277 (25.1)
No reconstitution needed	16 (8.8)	98 (8.9)
Change in coverage status favoring IDegAsp	14 (7.7)	82 (7.4)
Other	6 (3.3)	54 (4.9)

Data are number of patients (%). Physicians could select more than one reason for each patient. 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 patients in the full analysis set.

4.62 kg/m^2), and very poor glycemic control with mean HbA1c of 10.0% (SD 2.14%). Before initiating or switching to IDegAsp, 165 (90.7%) patients had received other anti-hyperglycemic therapies. The physicians' reasons for initiating or switching to IDegAsp are summarized in Table 2. The main reason behind initiating or switching to IDegAsp in the Malaysian cohort (92.9%) and the overall study population (93.1%) was to improve glycemic control. A higher proportion of patients (51.1%; n = 93) received OD regimen of IDegAsp versus the BID regimen (48.9%; n = 89) at treatment initiation. The mean (SD) initial total daily dose of IDegAsp was 29.1 (19.7) U.

Glycemic control

The HbA1c and FPG were significantly reduced from baseline to EOS (estimated difference [ED]: –1.3% [95% CI: –1.61 to –0.90]; p<0.0001 and ED: –1.8 mmol/L [95% CI: –2.49 to –1.13]; p<0.0001 respectively). The number of patients with HbA1c levels less than 7.0% increased from 10 (5.5%) at baseline to 25 (17.0%) at EOS.

Insulin dose

There was a reduction in the total daily dose of insulin in insulin-experienced patients (using prior basal insulin only, basal–bolus insulin and premixed insulin) at EOS compared with that at baseline (ED: –1.9 U [95% CI: –7.95 to 4.18]; p=0.5378). Similarly, a reduction in daily prandial insulin dose was observed in these patients (ED: –1.8 U [95% CI: –5.70 to 2.12]; p=0.3648). Likewise, the daily basal insulin dose was also reduced at EOS compared with baseline (ED: –0.6 U [95% CI: –2.90 to 1.67]; p=0.5931). However, these reductions were not statistically significant.

Hypoglycemia

The number of events and number of patients experiencing overall and nocturnal non-severe hypoglycemia and severe hypoglycemia in 4 weeks before IDegAsp initiation reduced from baseline (non-severe, 38; nocturnal, 19; severe, 7) to that within 4 weeks preceding EOS or discontinuation (non-severe, 11; nocturnal, 2; severe, 1; Table 3).

Body weight

A significant reduction in body weight was observed at EOS compared with baseline (ED: –0.9 kg [95% CI: –1.69 to –0.02]; p=0.046) in the Malaysian cohort. The same was also observed in the overall study population at EOS compared with baseline (ED: –1.0 kg [95% CI: –1.51 to –0.52]; p<0.0001).[16] On subgroup analysis of the overall population, a statistically significant reduction in body weight was observed in prior OAD-only users (ED: –1.4 kg [95% CI: –2.32;–0.49]; p=0.0028), basal insulin users (ED: –1.1 kg [95% CI: –2.09;–0.07]; p=0.0362), and basal–bolus insulin users (ED: –1.5 kg [95% CI: –2.70;–0.23]; p=0.0212). In patients who received GLP-1RA ± insulin treatment previously, (ED: 0.3 kg [95% CI: –1.10;1.77]; p=0.6411) a small increase in body weight was observed.[16]

Adverse events

Overall, 37 adverse events (AEs) were observed in 23 (12.6%) patients. Of these, 23 non-serious events were reported in 15 (8.2%) patients and 14 serious events in 11 (6.0%) patients. Further evaluation indicated that 10 serious and 18 non-serious events were unlikely to be caused by IDegAsp treatment. The AEs are shown in Table 4.

Healthcare resource utilization

Healthcare resource utilization in the 12-weeks before baseline and the 12-weeks prior to EOS or discontinuation in both global and Malaysian cohorts is shown in Table 5. The number of self-reported outpatient visits among the Malaysian cohort within the 12 weeks before baseline and within 12 weeks before EOS or discontinuation were 55 and 24, respectively. Within 12 weeks prior to treatment

Table 3. Hypoglycemic episodes occurring during 4 weeks prior to initiation of IDegAsp (baseline) and during 4 weeks prior to EOS or discontinuation (i.e., the last 4 weeks of the on-treatment period) during on-treatment observation period

	Malaysia N=182		Overall study N=1102	
	Number of events	Number of patients, n (%)	Number of events	Number of patients, n (%)
Non-severe hypoglycemic episodes				
Number of events/patients	49	23	526	163
Within 4 weeks prior to treatment initiation	38	21 (91.3)	364	128 (78.5)
Within 4 weeks prior to EOS or at discontinuation	11	3 (13.0)	162	44 (27.0)
Nocturnal non-severe hypoglycemic episodes				
Number of events/patients	21	12	173	72
Within 4 weeks of initiation	19	11 (91.7)	142	59 (81.9)
Within 4 weeks prior to EOS or discontinuation	2	1 (8.3)	31	14 (19.4)
Severe hypoglycemic episodes				
Number of events/patients	8	6	54	26
Within 26 weeks of initiation	7	5 (83.3)	51	23 (88.5)
Within 26 weeks prior to EOS or discontinuation	1	1 (16.7)	3	3 (11.5)

Data based on the FAS, on-treatment observation period; EOS, end of study; N, number of patients in the full analysis set; n, number of patients with response.

Table 4. Solicited serious and non-serious selected significant AEs during in-study observation period

	Malaysia N=182	
	Number of events	Number of patients, n (%)
Non-serious		
Number of events/patients	23	15 (8.2)
Severity		
Mild	17	12 (6.6)
Moderate	6	5 (2.7)
Severe	0	0
Serious		
Number of events/patients	14	11 (6.0)
Severity		
Mild	1	1 (0.5)
Moderate	5	5 (2.7)
Severe	8	6 (3.3)

Data based on the FAS, in-study observation period; N, number of patients in the full analysis set; n, number of patients with response.

initiation, eight patients reported having missed workdays due to diabetes and its complications, while none of the patients reported missed workdays after treatment initiation in the Malaysian cohort.

DISCUSSION

This subgroup analysis of the non-interventional real-world ARISE study was conducted to assess the glycemic control and various clinical outcomes related to the administration of IDegAsp in patients initiated or switched to IDegAsp therapy. IDegAsp resulted in significant improvements in glycemic control in the Malaysian cohort, as evident by a reduction in HbA1c levels from baseline to EOS. In addition, FPG was significantly reduced from baseline to EOS and the number of non-severe and severe hypoglycemic events also reduced from baseline following IDegAsp treatment.

The results from the current subgroup analysis are consistent with those reported in a meta-analysis of 17 studies comparing IDegAsp with insulin analogs. The meta-analysis included 3831 patients with T2DM, where IDegAsp BID significantly reduced FPG and minimized nocturnal hypoglycemia risk in comparison to conventional premixed insulin BID; however, both insulin types had a similar effect on HbA1c levels.[17] In another meta-analysis of six RCTs including 1346 patients with T2DM, a significant decrease in mean HbA1c was reported with IDegAsp OD compared with insulin glargine (IGlar) OD.[18] In another retrospective observational study, IDegAsp OD led to significantly lower HbA1c levels and FPG than basal insulin in 87 patients with T2DM in each treatment group.[19] The reduction in FPG with IDegAsp could be attributed to the long-acting effect of the IDeg analog while HbA1c reduction may be due to the prandial coverage of the IAsp analog.[17]

The doses of total daily, prandial and basal insulin were reduced from baseline to EOS in this study; however, these reductions were not statistically significant. A similar trend was observed in a retrospective real-world study in Japan where there was a significant reduction ($p<0.0001$) in daily basal insulin dose over 26 weeks in patients with T2DM who were administered with IDegAsp.[20] Also, nine studies in the meta-analysis reported a reduction or no difference in total dose requirement for IDegAsp compared to other basal insulins.[17]

In our study, the incidence of overall and nocturnal non-severe and severe hypoglycemic events was reduced from baseline to EOS. Although the low number of hypoglycemic events did not allow for statistical comparisons, these results suggest that improvement in glycemic control can be achieved with IDegAsp potentially without increased risk of hypoglycemia. In the meta-analysis of studies comparing IDegAsp and IGlar, the rates of confirmed overall hypoglycemia (odds ratio [OR] = 1.59; 95% CI: 0.97 to 2.61; p=0.07; I^2=66%) and nocturnal hypoglycemia (OR=0.54, 95% CI 0.31 to 0.94, p=0.49; I^2=57%) were not significantly different between the treatment groups.[18] Similarly, the risk of confirmed hypoglycemia with IDegAsp, premixed insulin BID (OR 0.52; 95% CI: 0.42 to 0.65; I^2=23.9%) and basal insulin OD (OR 0.51, 95% CI 0.27 to 0.95, I^2=66.0) was comparable. In a meta-analysis, nocturnal hypoglycemia was significantly reduced with IDegAsp.[17]

Table 5. Healthcare resource utilization during on-treatment observation period

HRU associated with diabetes and its complications	Malaysia N=182		Overall study N=1102	
	n	Mean (SD)	n	Mean (SD)
Self-reported outpatient visits				
Within 12 weeks prior to initiation	55	5.0 (16.66)	394	3.2 (6.77)
Within 12 weeks prior to EOS or discontinuation	24	1.7 (1.73)	195	2.5 (2.88)
Self-reported emergency room visits				
Within 12 weeks prior to initiation	8	1.4 (1.06)	46	1.3 (0.66)
Within 12 weeks prior to EOS or discontinuation	4	1.8 (0.50)	8	1.4 (0.52)
Self-reported other healthcare provider visits and contacts outside of the hospital setting (face-to-face, telephone and email)				
Within 12 weeks prior to initiation	5	1.4 (0.55)	69	2.4 (3.32)
Within 12 weeks prior to EOS or discontinuation	2	1.0	12	1.7 (1.72)
Self-reported workdays missed				
Within 12 weeks prior to initiation	8	15.8 (18.58)	58	8.7 (14.90)
Within 12 weeks prior to EOS or discontinuation	0	0	9	3.1 (3.10)
Self-reported in-patient hospitalizations				
Within 12 weeks prior to initiation	14	1.2 (0.58)	78	1.2 (0.43)
Within 12 weeks prior to EOS or discontinuation	4	1.3 (0.50)	12	1.7 (1.72)

Data based on FAS. EOS, end of study; HRU, healthcare resource utilization; N, number of patients in the FAS; n, number of patients with response.

IDegAsp also demonstrated better glycemic control in multinational patients with T2DM before, during and following Ramadan fasting in a randomized treat-to-target trial with a 74% reduction in overall hypoglycemia, 83% reduction in nocturnal hypoglycemia and 44% reduction in severe hypoglycemia compared with the premixed insulin analog, biphasic IAsp 30. These results suggest that IDegAsp could also be an appropriate choice of treatment for patients who fast for 12 to 16 hours daily during Ramadan in countries in Asia, Africa and the Middle East, including Malaysia.[21,22] A multicenter, prospective, post-marketing surveillance study found IDegAsp to have long-term safety, efficacy and tolerability in a Japanese real-world setting.[23] Another prospective real-world study on Japanese patients with T2DM reported similar rates of non-severe hypoglycemia before and after switching to IDegAsp from IGlar U100/U300, suggesting glycemic control, safety and tolerability to IDegAsp.[20]

Severe hypoglycemia is mostly seen in patients with T2DM who are more than 75 years of age.[23] In addition, hypoglycemic episodes are major concerns in insulin initiation and treatment intensification among patients and physicians.[5] Hence, hypoglycemic episodes can be a limiting factor for insulin intensification, particularly in elderly patients.[23] However, post hoc analysis of the 26-week BOOST clinical trial program which enrolled 756 patients from several countries including Malaysia, reported that IDegAsp BID was effective in improving glycemic control with reduced incidence of hypoglycemia in elderly patients with T2DM.[24]

In our study, a decrease in body weight from baseline to EOS (ED: –0.9 kg [95% CI –1.69 to –0.02], p=0.046) was observed, similar to that in the overall study population. Likewise, a 52-week trial on patients with T2DM reported a reduction in mean body weight by 0.78 kg in patients administered IDegAsp.[25] Weight loss and a significant decrease in BMI were also observed in patients on IDegAsp treatment for 12 months.[26] Taken together, all these studies have reported similar body weight changes with IDegAsp, as in this study.

Several studies have reported lower rates of AEs in patients on IDegAsp treatment regimen with hypoglycemia as the most frequent AE reported.[20,23] Although 37 AEs were recorded across 23 patients in the Malaysian cohort, most of them were judged unlikely to be caused by IDegAsp treatment. There were fewer HRU in terms of self-reported outpatient visits, visits to the emergency room and other healthcare providers, in-patient hospitalizations and missed workdays during the 12 weeks prior to EOS or discontinuation compared to 12 weeks before IDegAsp treatment initiation. However, the numbers were too small to draw firm conclusions.

This study provided insights into diabetes management in a real-world setting. A high number of patients completing the study, a larger cohort, and a multicenter study design revealed a robust dataset. The prospective study design with broad inclusion and exclusion criteria facilitated data collection from an optimal study cohort. However, the study findings might not be generalizable as only the Malaysian population was analyzed. Moreover, this was a single-arm, open-label study without a comparator group. Additionally, patients expected to benefit from a change of regimen to IDegAsp were selected by their physicians for the study, which could have led to potential selection bias.

CONCLUSION

The results from this analysis of a Malaysian cohort with T2DM who initiated or switched to IDegAsp in the real-world setting demonstrated improved glycemic control, reduced mean insulin dose in insulin-experienced patients and reduced frequencies of non-severe and severe hypoglycemic events. The findings from this study support the real-world use of IDegAsp in patients with T2DM who are not adequately controlled with non-insulin anti-hyperglycemic therapies.

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

CRediT Author Statement
MM: Investigation, resources, writing – review and editing, visualization, supervision; **SCL:** Investigation, resources, writing – review and editing, visualization; **MM:** Investigation, resources, writing – review and editing, visualization, supervision; **SU:** Conceptualization, investigation, resources, writing – original draft preparation; writing – review and editing, visualization, project administration, funding acquisition; **DM:** Writing – original draft preparation, writing – review and editing, project administration, funding acquisition; **MSMK:** Investigation, resources, writing – review and editing, visualization; **SS:** Investigation, resources, writing – review and editing, visualization; **AMD:** Investigation, resources, writing – review and editing, visualization; **KMC:** Investigation, resources, writing – review and editing, visualization; **LYT:** Investigation, resources, writing – review and editing, visualization; **SBN:** Investigation, resources, writing – review and editing, visualization; **JHGL:** Investigation, resources, writing – review and editing, visualization; **ZH:** Investigation, resources, writing – review and editing, visualization; **KBTSAK:** Investigation, resources, writing – review and editing, visualization; **BKL:** Investigation, resources, writing – review and editing, visualization; **SPC:** Investigation, resources, writing – review and editing, visualization.

Author Disclosure
All the authors have received honoraria from Novo Nordisk, unrelated to the current manuscript. The primary author is a member of the local advisory board for Novo Nordisk who received research contracts from Novo Nordisk.

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SUPPLEMENT

Supplementary Table 1. Reasons for discontinuing IDegAsp treatment during the study period

	Overall n=19
Insufficient effect on glycemic control	1 (5.3)
Unacceptable hypoglycemia profile/pattern	0
Lack of convenience	1 (5.3)
Adverse event	1 (5.3)
Change in coverage status disfavoring IDegAsp	6 (31.6)
Pregnancy or intentions to become pregnant	1 (5.3)
Weight gain	0
Other	9 (47.4)
Unknown	0

Footnote: Data are number of patients (%). Analyzed using the on-treatment observation period. A change in coverage status disfavoring IDegAsp refers to a change in healthcare insurance or reimbursement requirements that led to worse access to the drug. n, number of patients with response. 'Other' includes various reasons such as financial constraints, inter-district travel bans due to COVID-19, restrictions and concerns related to COVID-19 etc.

ORIGINAL ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

The Potential Relationship Between Serum Irisin Concentration With Inflammatory Cytokines, Oxidative Stress Biomarkers, Glycemic Indices and Lipid Profiles in Obese Patients With Type 2 Diabetes Mellitus: A Pilot Study

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Abstract

Objectives. Diabetes mellitus is a serious health-treated problem identified by disorders such as insulin resistance, dyslipidemia, and inflammation. Irisin, a newly discovered myokine/adipokine, is involved in metabolic homeostasis. The present study was carried out to investigate the potential relationship between serum irisin with inflammatory cytokines, oxidative stress biomarkers, glycemic indices, and lipid profiles in obese patients with type 2 diabetes mellitus.

Methodology. This analytical cross-sectional study was conducted on 62 participants (n=32 obese participants with diabetes, n=30 participants with normal weight). The participants answered a demographic questionnaire. Serum irisin, glycemic indices, lipid profiles, inflammatory cytokines and oxidative stress biomarkers were measured using standard methods. The difference between groups was assessed by independent-sample t-test or by a non-parametric equivalent. For qualitative variables, the Chi-Square test was used. Pearson rho coefficient was used to determine the potential relationship between irisin and inflammatory cytokines, oxidative stress biomarkers, glycemic indices, and lipid profiles. A $p<0.05$ was defined as significant.

Results. The median (IQR) age of the obese participants with diabetes was 54.0 years (52.2-60.7) and in the normal weight group was 38.0 years (30.0-47.2) ($p<0.001$). About 78% and 60% of participants in the obese with diabetes and the normal weight groups were females ($p>0.05$), respectively. Significant differences were observed in serum irisin levels between the two groups, with lower levels (218.74 ng/mL, [144.98-269.26]) noted in the obese with diabetes group compared to the normal weight group (266.68 ng/mL, [200.64-336.57]) with a $p=0.024$. There was a substantial difference between the two groups regarding IL-6, TNF-α, and hs-CRP ($p<0.05$). IL-6 had a moderate negative correlation with irisin in obese patients with T2DM (r=-0.478, $p=0.006$).

Conclusion. Irisin concentration was detected to be lower in obese people with diabetes. A negative relationship was detected between irisin and IL-6. Considering emerging evidence about the beneficial functions of irisin in improving metabolic abnormalities, designing future studies with greater sample sizes that will validate these results is needed.

Key words: Irisin, inflammation, glycemic indices, lipid profile, obesity, type 2 diabetes

INTRODUCTION

Diabetes mellitus is a severe global health problem and is one of the ten causes of morbidity and mortality in the adult population.[1,2] The prevalence of diabetes mellitus has increased in the past decades. In 2021, diabetes prevalence in all adults aged 20-79 years was estimated to be 10.5% worldwide (about 537 million).[3] It is estimated that this rate will rise to 783 million by 2045.[4]

In people with diabetes, insulin secretion is impaired or decreased due to beta-cell dysfunction. Glucose intolerance, hyperglycemia, insulin resistance and diabetes-related conditions such as dyslipidemia and inflammation are

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the main drivers of diabetes.[5] Cytokines released by adipose tissue initiate the pro-inflammatory status. This contributes to the downregulation of insulin signaling receptors in β-cells and insulin resistance.[6] One of the common co-existing conditions with diabetes mellitus is dyslipidemia, seen in. approximately 50% of people with diabetes.[7] The perturbation in lipid metabolism interacts with insulin resistance which may become contributory to the development of cardiovascular complications in type 2 diabetes mellitus (T2DM).[8]

The various microvascular and macrovascular complications of diabetes annually impose huge costs on the healthcare system.[9] In the long term, the complications of diabetes affect the quality of life of people with diabetes and reduce their life expectancy.[10] Thus, it is necessary to identify novel diagnostic and therapeutic approaches to timely diagnose diabetes and its related comorbidities.

Irisin, a proliferator-activated receptor-γ coactivator-1α (PGC-1α)-dependent myokine, is mainly secreted by skeletal muscles and exerts beneficial functions in human health. The PGC-1α expression influences irisin secretion in skeletal muscle cells. The expression of the PGC-1α protein promotes the expression of the transmembrane protein fibronectin type III domain-containing protein 5 (FNDC5), the precursor of irisin.[11] Aside from skeletal muscles, adipose tissue, liver, heart, brain, tongue, rectum, subcutaneous glands, stomach, spleen, and testis secrete irisin slightly.[12] The expression of FNDC5 in skeletal muscle is 100–200 times higher than in adipose tissue.[13]

Irisin exerts crucial effects on metabolic processes,[14] it modulates glucose homeostasis by enhancing glucose uptake by target cells, inhibits gluconeogenesis, increases insulin sensitivity and induces glucose transporter four (GLUT4) expression. Also, irisin imposes anti-inflammatory effects on macrophages and adipocytes.[15] A high level of irisin controls the levels of inflammatory cytokines such as IL-1β and TNF-α.[16]

Irisin exerts anti-oxidative and anti-apoptotic properties in various pathological conditions by enhancing the production of antioxidant enzymes and decreasing the production of reactive oxygen species.[17] Irisin promotes fatty acid oxidation,[18] increases the release of glycerol molecules and inhibits lipid accumulation in adipocytes by up-regulation of the expression of genes involved in lipolysis such as hormone-sensitive lipase (HSL), adipose tissue triglyceride lipase (ATGL), and fatty acid binding protein 4 (FABP4).[19]

The results of studies regarding circulating irisin were inconsistent. Reports revealed that there was a low level of irisin in patients with breast cancer, chronic kidney disease, chronic obstructive pulmonary disease, Behcet's disease, early-stage of non-alcoholic fatty liver disease (NAFLD) and hypothyroidism.[20,21] In contrast, it was previously observed that circulating irisin was high in individuals with obesity, late-stage NAFLD, polycystic ovary syndrome, coronary artery diseases, metabolic syndrome and gastrointestinal system cancer.[22,23] Also, controversial results were reported regarding the relationship between irisin and biochemical parameters in individuals with diabetes. Some studies showed a positive correlation between biochemical and metabolic factors, such as glycemic indices and anthropometric measurements in patients with T2DM with irisin. In contrast, others revealed a negative or no correlation.[24,25] There are limited human studies that assessed the relationship of irisin with inflammatory cytokines, oxidative stress biomarkers and biochemical factors. Given that there is insufficient evidence regarding the beneficial aspects of irisin in inflammatory and metabolical pathways, the present study aimed to determine the relationship between irisin and inflammatory cytokines, oxidative stress biomarkers, glycemic indices and lipid profiles in obese patients with T2DM.

METHODOLOGY

Study design and enrollment of participants

This analytical cross-sectional study was carried out on 62 participants (n=32 obese participants with diabetes, n=30 participants with normal weight).

Participants with diabetes enrolled in the study were referred from the healthcare centers of Maragheh University of Medical Sciences between August 2020 and March 2021. The volunteer healthy normal-weight participants were enrolled through recruitment announcements.

The protocol of the study was approved by the Regional Ethics Committee of the Maragheh University of Medical Sciences (Registration Number: IR.MARAGHEHPHC.REC.1398.029). Upon enrolment, the participants filled out a written consent form.

Participants were not included if they fulfilled any of the following criteria: age more than 65 and below 18 years, duration of diabetes history more than 6 months, severe mental and physical disabilities, co-existing medical conditions such as chronic diseases such as chronic kidney disease, type 1 diabetes, cirrhosis, autoimmune diseases and heart failure, pregnancy, lactating mothers, and patients taking insulin, anti-inflammatory agents, multivitamins or weight loss drugs.

Procedures

Participants were asked to accomplish a demographic questionnaire. The body mass index was calculated using weight (kilogram) and height (meter). Blood samples (approximately 5 ml) were collected in a fasting state. After serum extraction, samples were kept and frozen at -80 °C.

Samples were then processed accordingly for biochemical parameters which included the following: serum irisin concentration via enzyme-linked immunosorbent assay

(ELISA) test (Zellbio GmbH, Ulm, Germany [Inter-Assay: CV<12%, Intra-Assay: CV<10%]); serum malondialdehyde (MDA) and the total antioxidant status (TAS) via spectrophotometer using a commercial kit (Merck chemicals and Randox Laboratories, Ltd, Crumlin, UK, respectively); serum level of TNF-α and IL-6 using the ELISA method (Zellbio GmbH, Ulm, Germany [Inter-Assay: CV<12%, Intra-Assay: CV<10%] and E0082Hu, Bioassay Technology Laboratory, Shanghai, China [Inter-Assay: CV<10%, Intra-Assay: CV<8%]); high sensitive-C reactive protein level via immunoturbidimetry assay (Biosystems, Barcelona Spain COD 31927); serum levels of fasting blood sugar (FBS), triglycerides (TG), high-density lipoprotein – cholesterol (HDL-C), low-density lipoprotein – cholesterol (LDL-C), and total cholesterol (TC) via photometric method (Pars Azmoun Company, Tehran, Iran [Inter-Assay: CV<3.6%, Intra-Assay: CV<1%]); HbA1c level via commercial kit using the immunoturbidometric assay (Autoanalyzer, BT 1500, Biotecnica Instruments, Italy. The insulin level was measured via chemiluminescence method (Abbott ARCHITECT i2000SR, Chicago, USA).

To evaluate insulin resistance, the homeostatic model assessment insulin resistance (HOMA-IR) was computed by dividing the product of fasting insulin (FI) level (μU/ml) and fasting serum glucose level (mmol/L) by a factor of 22.5. The quantitative insulin sensitivity check index (QUICKI) model assessed insulin sensitivity using the following formula: QUICKI = $1/[\log(I_0) + \log(G_0)]$, where I_0 is insulin (μIU/ml), and G_0 is fasting serum glucose (mg/dl).[26] Estimated average glucose (eAG) was calculated using the formula: eAG = 28.7 x A1c– 46.7.

Sample size estimation

The results of Shanaki et al.'s study[27] were used for computing sample size. Because the median and IQR (1st and 3rd quartile) of irisin among diabetics and the healthy group have been reported in the cited study, the median and IQR (1st and 3rd quartile) values were converted to the mean and SD by using the proposed formulas in Xiang Wan et al., study as:

$$\text{Mean} = \frac{(q1+\text{Median}+q3)}{3} \quad \text{and SD} = \frac{(q3 - q1)}{1.35}$$

(Q1 = 1st quartile, Q3 = 3rd quartile, SD = Standard deviation)

The reported median and IQR (1st – 3rd quartile) of irisin among diabetes patients and healthy participants were 1.96 (1.32- 3.32) and 4.14 (2.7 – 6.34) which were then converted to mean (SD) of 2.2 (1.5) and 4.4 (2.7), respectively. The computed minimum sample size was 21 per group. This sample size was adjusted to at least 23 per group in consideration of a possible drop-out rate of 10%.

Statistical analysis

The normality of data was assessed by both Shapiro–Wilk and Kolmogorov-Smirnov tests. Normally distributed quantitative data were presented as means and standard deviations (SD) and non-normal distributed data were shown as medians (25 to 75 percentile). The qualitative data were presented as frequencies (percent). To evaluate the mean difference between both groups, the independent-sample t-test for normal variables and the Mann-Whitney U-test for non-normal variables was used. Qualitative variables between groups were examined by the Chi-square test. Pearson correlation coefficient (Pearson's r) was used to determine the potential relationship between irisin level with inflammatory biomarkers, glycemic indices and lipid profiles. A p<0.05 was defined as statistically significant.

Data were analyzed using Statistical Product and Service Solutions (SPSS) Version 21 (SPSS Inc., Chicago, IL).

RESULTS

The mean (SD) ages of participants in the obese diabetes group and the normal weight group were 54.0 years (52.2-60.7) and 38.0 years (30.0-47.2), respectively, with significant differences between the two groups (p <0.001). About 78% of participants in the obese-diabetes group and 60% in the normal weight group were females. The baseline characteristics of the participants are shown in Table 1.

The serum irisin level was significantly different between the two groups, with the obese diabetes group noted to be lower at 218.74 ng/mL (SD: 144.98-269.26]) compared to the normal weight group at 266.68 ng/mL (SD: 200.64-336.57) with a p=0.024. The FBS, HbA1c and eAG in the obese with diabetes group was 139.0 mg/dL (108.0-157.0), 7.4% (6.2-8.4) and 161 (131-193), respectively, and there were significant differences between the two groups for these variables. Moreover, there were also significant differences between groups for inflammatory biomarkers IL-6, TNF-α, and hs-CRP. The obese patients with T2DM had a higher level of TG than the normal weight group (p<0.001). The tabulated results of the biochemical parameters for both groups are shown in Table 2.

Table 1. The baseline characteristics of participants enrolled in the study (n=62)

Variables	Obese with diabetes (n=32)	Normal weight (n=30)	p
Age (year)[b]	54.0 (52.2-60.7)	38.0 (30.0-47.2)	<0.001
Educational level[c]			
Illiterate	22 (6.8)	2 (6.7)	<0.001
Diploma	9 (28.1)	4 (13.3)	
Bachelor's degree	1 (3.1)	24 (80.0)	
Occupation[c]			
Employee	5 (15.6)	25 (83.3)	<0.001
Housewife	27 (84.4)	5 (16.7)	
Sex[c]			
Male	7 (21.9)	12 (40.0)	0.122
Female	25 (78.1)	18 (60.0)	
BMI (kg/m^2)[a]	34.35 ± 4.07	24.33 ± 3.70	<0.001

[a] Data presented as mean (SD), Independent-samples t-test
[b] Data presented as Median (IQR), Mann-Whitney U-test
[c] Data presented as frequency (percent), Chi-Square test
p<0.05 was defined as significant

Table 2. The difference between biochemical parameters in obese participants with diabetes and normal-weight participants (n=62)

Variables	Obese with diabetes (n=32)	Normal weight (n=30)	*p* *
FBS (mg/dL)[b]	139.0 (108.0-157.0)	87.0 (83.0-89.0)	<0.001*
HbA1C (micro dL)[b]	7.4 (6.2-8.4)	5.3 (5.1-5.5)	<0.001*
eAG[b]	161.0 (131.0-193.0)	105.0 (102.2-111.0)	<0.001*
FI (μIU/mL)[b]	20.7 (9.9-28.8)	10.1 (7.1-14.4)	0.002*
HOMA-IR[b]	6.5 (3.0-9.7)	2.0 (1.7-3.0)	<0.001*
QUICKI[b]	0.29 (0.27-0.32)	0.33 (0.31-0.35)	<0.001*
IL-6 (ng/L)[a]	3.90 ± 1.44	2.27 ± 1.71	0.006*
TNF-α (ng/L)[a]	11.84 ± 3.26	9.85 ± 3.15	0.018*
hs-CRP (pm/mL)[b]	6.15 (3.50-9.45)	1.65 (1.20-2.20)	<0.001*
MDA (μmol/L)[a]	2.14 ± 0.78	1.88 ± 0.66	0.172
TAC (mmol/L)[a]	1.21 ± 0.31	1.14 ± 0.28	0.386
TG (mg/dL)[b]	157.0 (111.0-225.0)	92.0 (72.0-122.2)	<0.001*
TC (mg/dL)[b]	156.5 (139.7-182.7)	161.5 (138.2-184.2)	0.905
LDL-C (mg/dL)[b]	87.5 (73.2-108.5)	101.5 (82.2-118.0)	0.081
HDL-C (mg/dL)[b]	36.0 (36.0-41.5)	38.0 (35.7-44.2)	0.207

[a] Data presented as mean (SD), Independent-samples t-test.
[b] Data presented as Median (IQR), Mann-Whitney U-test
*p<0.05 was defined as significant
IL-6: Interleukin-6, TNF-α: Tumor necrosis factor alpha, hs-CRP: High-sensitivity C-reactive protein, MDA: Malondialdehyde, TAC: Total antioxidant capacity, FBS: Fasting Blood Sugar, eAG: estimated average glucose, FI: Fasting Insulin, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, QUICKI: Quantitative Insulin-Sensitivity Check Index, TG: Triglyceride, TC: Total Cholesterol, LDL-C: Low-Density Lipoprotein - Cholesterol, HDL-C: High-Density Lipoprotein - Cholesterol

Table 3 shows the relationship between irisin with inflammatory biomarkers, oxidative stress biomarkers, glycemic indices and lipid profiles between the two groups of participants. Assessing the relationship between irisin values and the different biochemical parameters, IL-6 showed a moderately negative correlation with irisin in obese patients with diabetes (r=-0.478, p=0.006, 95%CI [-0.749 to -0.185]). There was no significant correlation detected between irisin and other inflammatory biomarkers, oxidative stress, glycemic indices, and lipid profiles.

DISCUSSION

The present study demonstrated that the level of irisin in obese people with type 2 diabetes was significantly lower than in controls. A negative correlation was detected between irisin and IL-6 in obese patients with type 2 diabetes (r=-0.478, p=0.006). Previously published results regarding irisin's association with biochemical and metabolic biomarkers were inconsistent. In agreement with our study, Khorasani and company's study showed no significant correlation between irisin and FPG, HbA1C and HOMA-IR except for fasting insulin, TG, LDL-C, and HDL-C in 30 people with diabetes and CAD. On the other hand, the correlation between irisin and body mass index (BMI), age, and duration of disease was significant.[25] El Hadad et al., showed no significant difference in irisin levels in 60 patients with T2DM compared with 30 control patients. However, a substantial correlation was detected between irisin level and FBS, HbA1C, CRP, cholesterol, and TG.[28] He et al., demonstrated a significant correlation between irisin with HbA1C and LDL-C in 71 patients with T2DM and 40 normal control patients. Like our study, the irisin level was significantly lower in people with diabetes than in controls (p<0.001), and no significant correlation was observed between irisin and lipid parameters of TG and HDL-C.[29]

The low concentration of irisin in patients with T2DM could be attributed to the low level of PGC-1α expression.[30] Insulin resistance, inflammation via the induction of oxidative stress, and pro-inflammatory cytokines decrease the expression of FNDC5.[31,32] Oxidative stress, advanced glycated end-products and other toxins in chronic disorders also inhibit the secretion of irisin and irisin-related gene expression.[21] The different circulating concentrations of irisin in patients with T2DM, obese and normal weight subjects may be attributed to their body composition and abnormalities in their glucose and lipids.[33] In the absence of sufficient muscle mass in patients with abnormal blood glucose levels or T2DM, the expression of FNDC5/Irisin in skeletal muscles and adipose tissue is decreased.[24,34] The contradicting results between this study and that of others may be explained by the differences in the stage and duration of diabetes. In early diabetes, the elevated circulating irisin may constitute a compensatory response to decreased energy expenditure.[35] Other factors such as ethnicity, diet, genetic parameters, methodological variations, different assay kits used for irisin detection and differences in the studied population may influence irisin levels.[34]

Table 3. The relationship between Irisin level with inflammatory biomarkers, oxidative stress biomarkers, glycemic indices and lipid profiles in obese patients with diabetes and normal weights (n=62)

Variables	Groups		FBS	HBA1C	FI	eAG	HOMA-IR	QUICKI	IL-6	TNF-α	hs-CRP	MDA	TAC	TG	TC	LDL-C	HDL-C
Irisin (ng/mL)	Obese with diabetes	r	-0.192	-0.239	0.142	-0.240	0.087	-0.108	-0.478	-0.021	-0.309	-0.286	0.003	0.074	0.096	0.100	0.126
		p	0.292	0.187	0.438	0.186	0.635	0.555	0.006	0.910	0.085	0.113	0.988	0.688	0.688	0.587	0.493
		95% CI (Lower-Upper)	(-0.459 to 0.025)	(-0.486 to 0.031)	(-0.075 to 0.411)	(-0.486 to 0.032)	(-0.132 to 0.281)	(-0.344 to 0.269)	(-0.741 to -0.185)	(-0.365 to 0.313)	(0.587 to 0.027)	(-0.542 to -0.015)	(-0.340 to 0.336)	(-0.265 to 0.389)	(-0.288 to 0.440)	(-0.274 to 0.409)	(-0.254 to 0.443)
	Normal weight	r	-0.059	-0.144	0.108	-0.147	0.049	0.176	0.278	0.056	-0.140	0.028	-0.025	0.112	0.039	0.047	-0.178
		p	0.758	0.446	0.569	0.437	0.797	0.352	0.137	0.770	0.460	0.884	0.896	0.557	0.840	0.806	0.346
		95% CI (Lower-Upper)	(-0.395 to 0.310)	(-0.462 to 0.137)	(-0.192 to 0.454)	(-0.465 to 0.132)	(-0.262 to 0.388)	(-0.161 to 0.483)	(-0.271 to 0.651)	(-0.293 to 0.404)	(-0.390 to 0.198)	(-0.381 to 0.392)	(-0.365 to 0.380)	(-0.254 to 0.483)	(-0.417 to 0.440)	(-0.367 to 0.431)	(-0.501 to 0.206)

Pearson rho coefficients
** Correlation is significant at the 0.01 level
* Correlation is significant at the 0.05 level
FBS: Fasting Blood Sugar, eAG: estimated average glucose, FI: Fasting Insulin, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, QUICKI: Quantitative Insulin-Sensitivity Check Index, IL-6: Interleukin-6, TNF-α: Tumor Necrosis Factor - alpha, hs-CRP: High-sensitivity C-reactive protein, MDA: Malondialdehyde, TAC: Total antioxidant capacity, TG: Triglyceride, TC: Total Cholesterol, LDL-C: Low-Density Lipoprotein - Cholesterol, HDL-C: High-Density Lipoprotein - Cholesterol

In our study, there was a negative correlation between irisin and IL-6. Similar to irisin, IL-6 is a contraction-regulated myokine that is known to be a muscle-derived protein.[36] Limited human studies have assessed the relationship between irisin and inflammatory cytokines and oxidative stress. According to Liu et al., exposure of mice with type 1 diabetes to irisin at 0.5 to 1.5 μg/g body weight for 16 weeks remarkably decreased the production of ROS, MDA, IL-1β and IL-18.

Irisin exerts anti-inflammatory effects by suppressing the NLR family pyrin domain containing 3 (NLRP3) inflammasome, a mediator of cellular damage and inflammation. NLRP3 plays a critical role in the activation of caspase-1 that involves induction and secretion of pro-inflammatory cytokines such as pro-IL-18 and pro-IL-1β.[37,38] In the study of Shao et al., the lipopolysaccharide (LPS)-induced acute lung injury in a mice model that was treated with irisin revealed that irisin significantly decreased the production of the pro-inflammatory cytokines IL-6, MCP-1, IL-1β, and TNF-α. Irisin also reduced MAPK activation by LPS and nuclear factor (NF)-$_k$B signaling pathways in mice models and A549 cells.[39] Sanchis-Gomar noted that despite the lower level of irisin in obese patients with T2DM (n=34) than in controls (n=20), there was no significant difference between them. Moreover, a negative correlation between irisin and HbA1C (r=-0.401, *p*=0.025) and homocysteine (r=- 0.430, *p*=0.020) was noted in obese patients with T2DM.[40]

The primary mechanism attributed to the immune system boosting and anti-inflammatory properties of irisin is its role in decreasing the production of ROS and inhibiting the expression of inflammatory cytokines such as IL-6, TNF-α, and cyclooxygenase 2.[41] Irisin also inhibits the expression of toll-like receptor 4 (TLR4) and down-regulates the signaling pathway of mitogen-activated protein kinases (MAPK). Irisin suppresses the activation of NF-κB and IL-1β in the secretion of other pro-inflammatory cytokines, such as IL-8 and MCP-1, and causes the natural immune system to weaken.[42] It can also modulate inflammation by increasing the gene expression of CD206 and IL-10, which are the macrophage markers with anti-inflammatory properties, and upregulates NO synthesis.[43,44]

The other function of irisin is attributed to its anti-apoptotic effect. In people with diabetes, high glucose levels induce cell apoptosis, insulin insensitivity and impaired β-cell function.[45] Irisin downregulates the expression of apoptotic markers such as Bax, Bad, Caspase 9 and Caspase 3; in contrast, it enhances the expression and activity of anti-apoptotic markers such as Bcl-2 and Bcl-xl.[17] Irisin exerts anti-inflammatory effects indirectly via modulating the leptin level which is an insulin-sensitizing hormone.[13] It seems that the significant relationship between irisin and IL-6 compared with other inflammatory biomarkers can be attributed to the high sensitivity and rapid generation and response of IL-6, especially in the early phase of inflammation. As a pleiotropic cytokine, IL-6 exerts

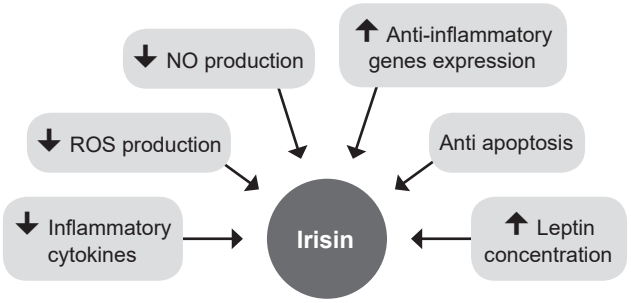

Figure 1. The potential anti-inflammatory role of Irisin in the management of chronic diseases.

fundamental functions in regeneration, immune system response and tissue repair.[46,47] Also, IL-6 is involved in the activation of other inflammatory signaling pathways such as CRP.[48] Figure 1 summarizes the potential anti-inflammatory role of irisin in the management of chronic diseases.

To the best of our knowledge, this study was the first to investigate the relationship between irisin concentration, inflammatory cytokines and oxidative stress biomarkers aside from glycemic indices and lipid profile parameters in patients with T2DM. However, this study had some limitations. Major limitations noted were the small sample size and the failure to measure other biomarkers and molecular pathways that may influence irisin levels in patients with diabetes. The lack of assessment of the participants' physical activity and body composition was considered another limitation. It is suggested that future studies be conducted to find the exact role of irisin in boosting the immune system and its anti-inflammatory properties to decrease the burden of disorders with low inflammation states.

CONCLUSION

In conclusion, the present study investigated the relationship between irisin and inflammatory biomarkers, glycemic indices and lipid profiles. The level of irisin was significantly lower in obese patients with T2DM than in healthy normal weight participants, and a significant negative correlation was detected between circulatory irisin and IL-6. Other biochemical factors did not show a substantial correlation with irisin. The potential protective functions of irisin in boosting the immune system and regulating metabolism in chronic disorders remains an open question that merits further investigations with a large-scale sample size to discover unknown mechanisms and other factors that affect circulating irisin in patients with T2DM.

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

CRediT Author Statement

YK: Conceptualization, Methodology, Writing – original draft preparation, Writing – review and editing, Supervision, Project administration; **AHF, SD:** Resources, Writing – original draft preparation; **AS:** Validation, Formal analysis, Data curation; **SI, SP:** Investigation, Resources; **LP:** Conceptualization, Methodology, Writing – review and editing, Supervision, Project administration, Funding acquisition.

Author Disclosure

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ORIGINAL ARTICLE

The Prevalence of Advanced Liver Fibrosis Among Patients With Type 2 Diabetes Mellitus: A Single-Centre Experience in Penang, Malaysia

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Abstract

Objectives. Type 2 diabetes mellitus (T2DM) is an important risk factor for Non-alcoholic fatty liver disease (NAFLD). It worsens the course of NAFLD. We investigated the prevalence of advanced liver fibrosis among patients with T2DM. Our secondary objectives were to describe patient demographics, to explore associated clinical factors, and to compare FIB-4 Index and liver stiffness measurement (LSM).

Methodology. This was a cross-sectional study on 258 patients with T2DM duration of at least 10 years. Transient elastography (FibroScan®) was performed on all subjects. Advanced liver fibrosis was diagnosed based on LSM results. The FIB-4 index formula was used.

Results. The prevalence of advanced liver fibrosis was 22.1%. Associated factors were body mass index (BMI), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), triglyceride (TG) and high-density lipoprotein (HDL) cholesterol. Independent factors were BMI and GGT (p=0.003 and p<0.001). FIB-4 index has 30.0% sensitivity, 85.0% specificity, 38.7% positive predictive value, and 79.4% negative predictive value in detecting advanced liver fibrosis by LSM criteria.

Conclusion. Our study confirmed the high prevalence of advanced liver fibrosis among patients with long-standing T2DM. This study suggests the benefit of advanced liver fibrosis screening in patients with a minimum of 10 years of T2DM, especially those with high BMI and GGT.

Key words: type 2 diabetes mellitus, non-alcoholic fatty liver disease, advanced liver fibrosis, transient elastography, FIB-4 index

INTRODUCTION

NAFLD and T2DM regularly co-exist and act synergistically to drive adverse outcomes. The presence of both NAFLD and T2DM increases the likelihood of the development of complications of diabetes as well as augments the risk of more severe NAFLD, including cirrhosis, hepatocellular carcinoma, and death. The mainstay of NAFLD management is currently to reduce modifiable metabolic risk factors. Achieving good glycaemic control and optimizing weight loss are pivotal to restricting disease progression.[1]

NAFLD is the most common chronic liver disease, affecting 15-40% of the population worldwide.[2] Around 20-30% of patients with NAFLD have non-alcoholic steatohepatitis (NASH), the active form of NAFLD which can cause liver fibrosis. This may eventually progress to cirrhosis and hepatocellular carcinoma in 10-20% of patients.[3-5] In the United States, NASH has already emerged as the second leading aetiology of chronic liver disease among new liver transplant registrants,[6] and is also the second leading cause of hepatocellular carcinoma.[7]

The prevalence of NAFLD and advanced liver fibrosis is high among patients with T2DM. In 2018, a Malaysian study of 571 patients with T2DM by Lee-Lee Lai found the prevalence of transient elastography-diagnosed NAFLD and advanced liver fibrosis to be 72.4% and 21.0% respectively,[8] whereas Kwok found the respective prevalence to be 72.8% and 17.7% in Hong Kong in 2016.[9]

Major guidelines have different recommendations with regards to screening for NAFLD among patients with T2DM. The European Association for the Study of the Liver (EASL) guidelines recommend screening patients with T2DM for NAFLD regardless of serum liver enzyme

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level in view of their high risk for disease progression.[10] On the other hand, the American Association for the Study of Liver Diseases (AASLD) guideline is not in favour of routine screening for NAFLD in patients with T2DM, citing uncertainties surrounding diagnostic tests and treatment options, and the lack of knowledge related to the long-term benefits and cost-effectiveness of screening.[11] Liver biopsy is the gold standard for the assessment of liver fibrosis, but it has its limitations, which include life-threatening complications. Alternative methods of non-invasive laboratory and radiologic testing for the assessment of liver fibrosis in NAFLD have evolved during the past decade, and these methods may be able to overcome the limitations of liver biopsy.[12,13]

An ultrasound-based technique, transient elastography (FibroScan®) is one of the most extensively used and well-validated non-invasive methods for the assessment of liver fibrosis.[14-18] recent meta-analysis showed that transient elastography had a high sensitivity of 94% and specificity of 95% when used to identify fibrosis in patients with NAFLD.[14] However, up to 20% of transient elastography examinations yielded unreliable results, especially among patients with high BMI.[19,20] The use of the XL probe can increase the success rate of examination in obese patients, but proper training is required.[21] To improve test reliability, a minimum of 10 valid readings, with at least a 60% success rate and an interquartile range of ≤30% of the median value, are taken with the results expressed in kilopascals (kPa).[15,22]

We are aware that it is impossible to perform FibroScan® routinely on all patients with long-standing T2DM. Only three tertiary public hospitals in the country offer FibroScan® for free. If done in a private hospital, the charges can amount to 800 Malaysian Ringgit. Hence, various scoring systems of fibrosis have been explored.

In 2009, Shah AG et al., concluded that the FIB-4 index [(Age x AST) / (Platelet x √(ALT)] is superior to 7 other non-invasive markers of fibrosis in patients with NAFLD, namely NAFLD Fibrosis score, Goteburg University Cirrhosis Index, AST:ALT ratio, AST:Platelet ratio index, AST:Platelet ratio, BMI, AST: ALT, diabetes (BARD) score and cirrhosis discriminant score. Their study used a nationwide database of 541 adults with NAFLD; jack knife-validated areas under receiver operating characteristic curves (AUROC) of FIB-4 and 7 other markers were compared. All patients in this dataset had a liver biopsy in the 12 months prior to enrolment.[16]

Data on the prevalence of NAFLD among patients with T2DM in the region of Southeast Asia is lacking. Availability of this data will help to assess the benefits and cost-effectiveness of NAFLD screening among patients with T2DM in this region and worldwide.

Our primary objective was to investigate the prevalence of advanced liver fibrosis by transient elastography among patients with at least 10 years of T2DM. We also aimed to describe the demographic and clinical profiles of the patients with advanced liver fibrosis, explore the factors associated with advanced liver fibrosis among patients with T2DM, and compare FIB-4 Index and LSM on FibroScan®.

METHODOLOGY

Subjects

Patients seen at the diabetes specialist clinic of the endocrinology unit in Penang General Hospital, Malaysia who were at least 35 years old and had long-standing T2DM for at least 10 years were enrolled after they provided written informed consent. Excluded were those with significant alcohol intake (greater than 21 units per week for males and greater than 14 units per week for females); established history of other forms of liver diseases including hepatitis B (positive serum hepatitis B surface antigen), hepatitis C (positive anti-hepatitis C antibody), autoimmune hepatitis (positive autoimmune serology with consistent biopsy result), drug-induced liver disease (history of amiodarone or tamoxifen use), and biliary duct obstruction; history of gastrointestinal bypass or use of drugs known to cause hepatic steatosis (i.e., amiodarone, valproate, tamoxifen, methotrexate, steroids); established history of liver cirrhosis; active substance abuse; history of platelet disorders; congestive cardiac failure who may have secondary liver congestion; presence of a pacemaker (according to FibroScan® manufacturer advice) and those who were pregnant.

Materials and methods

This was a cross-sectional prevalence study which took place from July 2019 to January 2020.

Before each diabetes specialist clinic consult, the subjects were screened and selected based on the study inclusion and exclusion criteria. On the actual visit, anthropometric measurements and vital signs (weight, height, waist circumference, and blood pressure) were taken at the registration counter by a designated nurse. During their consultation with the attending doctors, eligible patients were asked if they were keen to participate. Patients who agreed were sent to the study procedure room to meet the primary investigator after their consultation. The primary investigator would then give verbal and written explanations based on the patient information sheet. Informed consent was obtained and appointment dates for blood sampling and FibroScan® were given.

A total of 321 patients were recruited. Data collection was done based on the Data Collection Sheet. Venous blood samples were obtained at the Penang General Hospital outpatient clinic after a 10-hour overnight fast. Blood was sent for complete blood count, renal profile, liver function tests, fasting blood sugar, fasting lipid profile, glycosylated haemoglobin, aspartate aminotransferase and gamma-glutamyl transpeptidase. This step was omitted if latest

available results were performed not more than 4 months prior to recruitment.

Transient elastography

After blood extraction, the patients proceeded to the gastroenterology clinic for transient elastography using Fibroscan® 502 keyboard (Echosen™, Paris, France). This was a non-invasive imaging done to assess the severity of liver fibrosis. Transient elastography was performed by a single operator with either the M or the XL probe. If a patient failed to obtain a valid result with the M probe due to central obesity, the elastography was repeated using the XL probe.

Adequate pressure of the probe on the skin surface, good layering on TM mode, and a straight imaginary line on A mode were ensured for each measurement. An examination was considered successful if at least 10 valid measurements were obtained, and reliable if the interquartile range (IQR) / median of the LSM was at most 30%.[17] A patient was considered to have advanced fibrosis if the LSM was at least 9.6 kPa using the M probe or at least 9.3 kPa using the XL probe. Cirrhosis is considered if the LSM was at least 11.5 kPa using the M probe or at least 11.0 kPa using the XL probe.[20,21]

The FibroScan® available in our institution could only measure LSM. The machine is unable to measure controlled attenuation parameters (CAP). Hence, we were able to investigate the prevalence of advanced liver fibrosis but not hepatic steatosis.

Transient elastography reports were interpreted based on the following scoring card used by Echosens™, Paris, France (Figure 1).

Results were then grouped into

- F0: Normal
- F1: Mild fibrosis
- F2: Significant fibrosis
- F3: Severe fibrosis
- F4: Cirrhosis

Patients with a fibrosis score of F1 were offered two annual transient elastography surveillance. Patients with a fibrosis score of F2 were offered yearly assessment. Those who scored F3 and above were referred to a gastroenterologist for further assessment and surveillance of cirrhosis and hepatocellular carcinoma.

FIB-4 index

As transient elastography is not readily available in many parts of the world, scoring systems of liver fibrosis are important to assess the risk of fibrosis and the indication for this scan. We have chosen the FIB-4 Index as a scoring system option to calculate the risk of liver fibrosis in our subjects. This study compared FIB-4 Index to LSM on FibroScan®.

FIB-4 Index was calculated using the following calculator.[25]

$$\text{FIB-4} = \frac{\text{Age (years)} \times \text{AST Level (U/L)}}{\text{Platelet Count } (10^9/L) \times \sqrt{\text{ALT (U/L)}}}$$

For a fixed specificity of 90% (FIB-4 equal to 1.93), the sensitivity in identifying advanced fibrosis was only 50% (95% CI, 46-55%). A FIB-4 greater than or equal to 2.67 had an 80% positive predictive value and a FIB-4 index less than or equal to 1.30 had a 90% negative predictive value. Using the threshold values of 1.30 and 2.67 for the absence

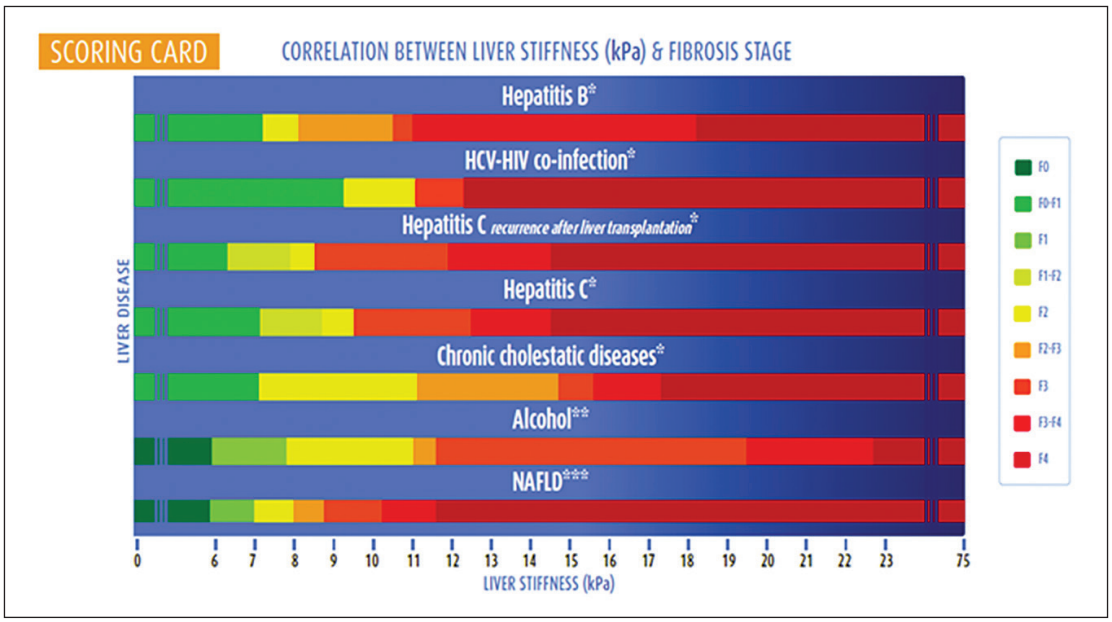

Figure 1. Scoring card for correlation between liver stiffness (kPa) and fibrosis stage (© Echosens™, Paris, France).[18,20,23,24]

and presence of advanced fibrosis, respectively, the FIB-4 index showed 89% accuracy. The FIB-4 index is to be used with caution in patients less than 35 or greater than 65 years old, as the score has been shown to be less reliable in these patients.[16]

Sample size calculation

Through literature search conducted on PubMed, we found six studies looking into the prevalence of liver fibrosis among diabetic patients using FibroScan®. The two studies that we selected were from Malaysia and Hong Kong (Table 1). These studies presumably had a more similar demographic profile with our patients and used the same cut-off values of FibroScan®. Other studies used different FibroScan® cut-offs and studied a different population group.[26-29] Hence, these studies were not considered in our sample size calculation.

Table 1. Two studies selected as references for sample size calculation[8,9]

Authors	Year	Population	Prevalence of liver fibrosis (%)	Sample size calculated
Lee et al.	2018	571 patients with T2DM in Malaysia	21.0	255
Kwok et al.	2016	1918 patients with T2DM in Hong Kong	17.7	224

We computed the sample size with 80% certainty (power) and alpha of 0.05. The calculation is based on the formula for sample size without finite population correction.[30]

$n = Z^2 P(1-P) / d^2$ where,

n = sample size

Z = Z statistic for a level of confidence = 1.96 for 95% level of confidence

P = Expected prevalence = 0.21

d = Precision = 0.05

Based on the study by Lee et al., we needed to include at least 255 patients. Accounting for an expected 20% drop-out rate, we planned to recruit a minimum of 319 patients.

Statistical analysis

The data analysis was done using SPSS version 22. Descriptive data were expressed as mean ± standard deviation (SD) unless otherwise stated. For demographic comparisons between patients with and without advanced liver fibrosis, the Chi-square or Fisher exact test was used for categorical variables, and independent *t*-test or Mann-Whitney U test was used for differences between continuous variables. Pearson correlation coefficient was used to evaluate correlations between LSM and FIB-4 Index.

Ethical Statement

The study was listed in the Malaysian National Medical Research Register (NMRR) (reference number NMRR-19-654-46941). The study protocol was approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia.

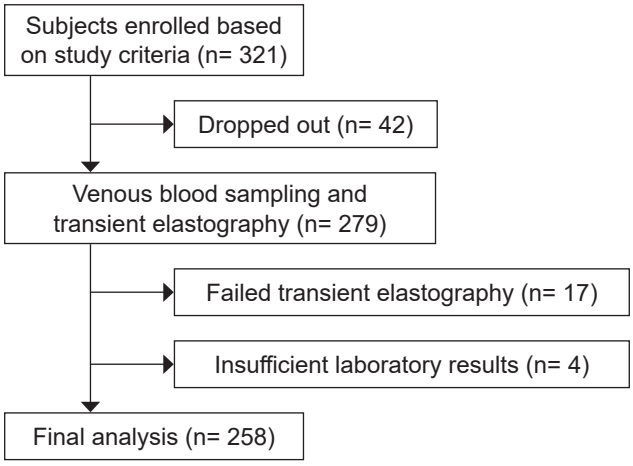

Figure 2. Study participant flow.

RESULTS

A total of 321 patients were recruited. Forty-two (13%) patients dropped out after recruitment for various reasons, 279 patients underwent venous blood sampling and transient elastography, 17 (6%) patients were unable to obtain valid results on transient elastography and 4 had insufficient results to proceed with further data analysis. Data from 258 patients were used for our final analysis (Figure 2).

Overview of the study population

The mean age of the 258 patients included in the analysis was 61.64 ± 10.35 years old. More than half were female (n=135, 52.3%). In the cohort, 38.0% were Chinese (n=98), 30.2% were Malays (n=78) and 28.7% were Indians (n=74). The more common associated co-morbidities were hypertension (n=188, 72.9%), ischemic heart disease (IHD) (n=67, 26.0%) and chronic kidney disease stage 3 and above (n=69, 26.7%).

The anthropometric indices showed that 21.3% of the subjects (n=55) were obese with a mean BMI of 27.04 ± 4.11 kg/m^2. The majority had central obesity (n=226, 87.6%) with a mean waist circumference of 95.69 ± 9.84 cm (Table 2).

Laboratory assessment

The median fasting blood sugar was 8.0 mmol/L and mean glycated hemoglobin was 8.3%. Median triglyceride (1.4 mmol/L), LDL cholesterol (2.1 mmol/L) and HDL cholesterol (1.2 mmol/L) levels were normal. Six patients had incalculable LDL cholesterol values because their triglyceride levels were more than 4.5 mmol/L (Table 2).

Prevalence of advanced liver fibrosis and associated factors

The prevalence of advanced fibrosis based on transient elastography was 22.1% (57 out of the 258 patients) (Table 3). Using simple logistic regression, the factors associated

Table 2. Baseline demographic and clinical profiles of the 258 subjects

Variables	n (%)	Mean ± SD
Age, in years		61.64 ± 10.35
Gender		
Male	123 (47.7)	
Female	135 (52.3)	
Ethnicity		
Chinese	98 (38.0)	
Malay	78 (30.2)	
Indian	74 (28.7)	
Others	8 (3.1)	
Hypertension	188 (72.9)	
Blood pressure control		SBP 134.68 ± 19.28 DBP 71.95 ± 9.29
Ischemic heart disease	67 (26.0)	
eGFR		
≥60 ml/min/1.73m^2	189 (73.3)	
<60 ml/min/1.73m^2	69 (26.7)	
BMI (kg/m^{-2}) †		27.04 ± 4.11
<30 kg/m^{-2}	203 (78.7)	
≥30 kg/m^{-2}	55 (21.3)	
Central obesity	226 (87.6)	
WC (cm)		95.69 ± 9.84
Male		97.05 ± 9.39
Female		94.46 ± 10.10
FBS (mmol/L) †		8.0 (5.15)
HbA1c (%)		8.3 ± 1.6 (67 ± 18)
Platelet count (10^9/L) †		269.50 (79.25)
Albumin (g/L)		37.97 ± 3.31
ALT (U/L) †		21.0 (16.0)
AST (U/L) †		20.0 (11.0)
GGT (U/L) †		29.0 (26.0)
TC (mmol/L) †		4.0 (1.2)
TG (mmol/L) †		1.40 (0.8)
LDL (mmol/L) †$		2.10 (1.0)
HDL (mmol/L) †		1.20 (0.4)

$ Missing values: LDL, 6. n=252
† Presented as median (IQR)
SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; WC, waist circumference; FBS, fasting blood sugar; HbA1c, glycated haemoglobin; TC, total cholesterol; LDL, low-density lipoprotein cholesterol

with advanced liver fibrosis were BMI, ALT, AST, GGT, TG, and HDL (Table 4).

Figure 3 shows the proportion of patients with advanced liver fibrosis based on different BMI cut-offs. For BMI greater than or equal to 23.0, 25.1% of patients who are overweight have advanced liver fibrosis. If the BMI cut-off is set at 27.5 kg/m^2 based on Malaysian obesity guidelines, 27.4% of patients have advanced liver fibrosis. However, when the BMI cut-off is set at 30 kg/m^2 based on WHO guidelines, the percentage of patients with advanced liver fibrosis increased to 38.2%.

By multiple logistic regression analysis, independent factors associated with advanced fibrosis were BMI and GGT (p=0.003 and p<0.001 respectively). Patients who were obese by WHO definition are 3.14 times more likely to develop advanced liver fibrosis (95% CI, 1.49 - 6.61). Patients who have elevated GGT are 8.39 times more likely to develop advanced liver fibrosis (95% CI, 4.20 - 16.78). This model predicted 81.7% of cases correctly with 68.2% sensitivity and 83.0% specificity. The model did not show any multicollinearity or interaction. Hosmer-Lemeshow test was not significant (p=0.967).

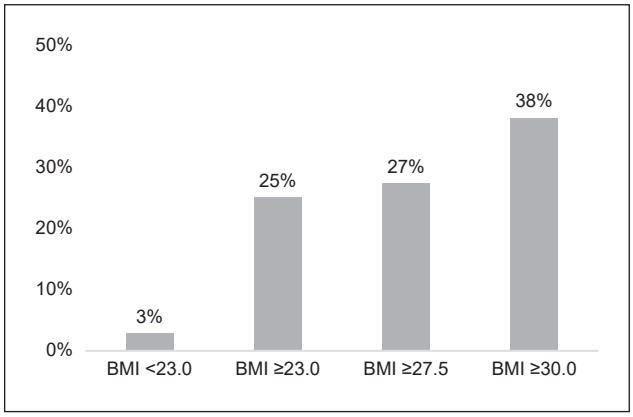

Figure 3. The proportion of patients with advanced liver fibrosis based on different BMI cut-offs.

FIB-4 index for the diagnosis of advanced liver fibrosis

The FIB-4 index should be used with caution among patients less than 35 or greater than 65 years old, as the score has been shown to be less reliable in these patients in the study by Shah et al.[20] We only included patients who are 35 years old and above. Including only the patients between 35 to 65 years old with a FIB-4 index cut-off of 1.31, the sensitivity, specificity, positive predictive value, and negative predictive value in detecting advanced liver fibrosis by LSM criteria were 30.0%, 85.0%, 38.7% and 79.4% respectively.

Serum ALT level for the diagnosis of advanced liver fibrosis

For the diagnosis of advanced liver fibrosis according to the WHO criteria, ALT cut-off was set at 30 U/L for males and 19 U/L for females. In our cohort, the combined sensitivity, specificity, positive predictive value, and negative predictive value of serum ALT were 64.9%, 63.7%, 33.6% and 86.5%, respectively.

DISCUSSION

In our cohort who had T2DM for at least 10 years, 22.1% had increased LSM suggestive of advanced liver fibrosis.

To date, there are two similar studies done in Southeast Asia. In 2016, a study published in Hong Kong recorded a prevalence of advanced liver fibrosis of 17.7%,[9] while another study done in Malaysia in 2018 recorded a prevalence of 21.0%.[8] The study cohort in Hong Kong had better glycaemic control (HbA1c 7.4%; FBS 7.4 mmol/L) compared to our cohort (HbA1c 8.3%; FBS 8.0 mmol/L). Their population had a mean waist circumference of 92.9cm, whereas ours had a mean waist circumference of 95.7cm. The BMI, cholesterol and blood pressure control were similar.

Table 3. Characteristics of patients with and without advanced fibrosis by Fibroscan®

Variables	Patients with advanced fibrosis, n=57		Patients without advanced fibrosis, n=201		*p*[b]
	Mean ± SD	n (%)	Mean ± SD	n (%)	
Age, in years	61.1 ± 8.6		61.8 ± 10.8		0.657
Gender					0.340
Female		33 (57.9)		102 (50.7)	
Male		24 (42.1)		99 (49.3)	
Ethnicity					0.651[c]
Chinese		19 (33.3)		79 (39.3)	
Malay		21 (36.8)		57 (28.3)	
Indians		16 (28.1)		58 (28.9)	
Others		1 (1.8)		7 (3.5)	
Systolic blood pressure	137.1 ±17.8		134.0 ± 19.7		0.283
Diastolic blood pressure	72.5 ± 8.7		71.8 ± 9.5		0.599
Blood pressure control					0.780
Controlled		34 (59.6)		124 (61.7)	
Uncontrolled		23 (40.4)		77 (38.3)	
Ischemic heart disease (Yes)		10 (17.5)		57 (28.4)	0.100
Serum creatinine, in mmol/L	78.0 (28.5) †		81.0 (34.0) †		0.822[d]
eGFR					0.673
≥60 ml/min/1.73m^2		43 (75.4)		146 (72.6)	
<60 ml/min/1.73m^2		14 (24.6)		55 (27.4)	
Insulin usage (Yes)		45 (78.9)		147 (73.1)	0.375
BMI, in kgm^{-2}					0.001
<30 kg/m^2		36 (63.2)		167 (83.1)	
≥30 kg/m^2		21 (36.8)		34 (16.9)	
WC, in cm	99.7 ± 10.1		94.6 ± 9.5		<0.001
Central Obesity[#]					0.162
No		4 (7.0)		28 (13.9)	
Yes		53 (93.0)		173 (86.1)	
FBS, in mmol/L					0.207
≤7.0		18 (31.6)		82 (40.8)	
>7.0		39 (68.4)		119 (59.2)	
HbA1c, in %					0.106
≤6.5		3 (5.3)		26 (12.9)	
>6.5		54 (94.7)		175 (87.1)	
Platelet Count, in x 10^9/L					0.124[c]
<150		2 (3.5)		1 (0.5)	
≥150		55 (96.5)		200 (99.5)	
Albumin in g/L					0.750
<35		8 (14.0)		25 (12.4)	
35-52		49 (86.0)		176 (87.6)	
ALT, in U/L[*]					<0.001
Normal		20 (35.1)		128 (63.7)	
Abnormal		37 (64.9)		73 (36.3)	
AST, in U/L					<0.001
<32		37 (64.9)		179 (89.1)	
≥32		20 (35.1)		22 (12.9)	
GGT, in U/L					<0.001
<40		16 (28.1)		156 (77.6)	
≥40		41 (71.9)		45 (22.4)	
TC, in mmol/L					0.273
<5.2		51 (89.5)		168 (83.6)	
≥5.2		6 (10.5)		33 (16.4)	
TG, in mmol/L					0.009
≤1.7		30 (52.6)		143 (71.1)	
>1.7		27 (47.4)		58 (28.9)	
LDL in mmol/L[$]					0.375
≤2.6		45 (83.3)		154 (77.8)	
>2.6		9 (16.7)		44 (22.2)	
HDL in mmol/L[&]					0.012
Normal		26 (45.6)		129 (64.2)	
Abnormal		31 (54.4)		72 (35.8)	

[a] The diagnosis of advanced fibrosis was based on LSM ≥9.6 kPa using the M probe or ≥9.3 kPa using the XL probe
[b] All variables were analysed using chi square tests (if categorical) or Student t-tests (if continuous) unless stated otherwise
[c] The variable was analysed using Fisher exact test
[d] The variable was analysed using Mann-Whitney U test as it is non-parametric
† Median (Interquartile range)
[#] Waist circumference: Male ≥90 cm; Female ≥80 cm
[*] ALT: normal: <30 U/L Male; <19 U/L Female. Abnormal: ≥30 U/L Male; ≥19 U/L Female
[$] Missing values: LDL, 6. n=252
[&] HDL: normal: >1.0 mmol/L Male; >1.2 mmol/L Female. Abnormal ≤1.0 mmol/L Male; ≤1.2 mmol/L Female

Table 4. Variables associated with the presence of advanced fibrosis by Fibroscan® using simple logistic regression and multiple logistic regression

Variable	Simple logistic regression				Multiple logistic regression		
	Crude OR	95% CI	X^2 stat (df)	*p*	Adjusted OR	95% CI	*p*
Age, in years	0.994	0.97 - 1.02	0.198 (1)	0.656			
Gender			0.913 (1)	0.341			
Male	1.000	ref					
Female	1.335	0.74 - 2.42					
Ethnicity			1.934 (3)	0.597			
Chinese	1.000	ref					
Malay	1.532	0.76 – 3.11		0.238			
Indian	1.147	0.54 – 2.42		0.719			
Others	0.594	0.07 – 5.12		0.636			
BP Control			0.078 (1)	0.780			
Controlled	1.000	ref					
Uncontrolled	1.089	0.60 – 1.99					
IHD			2.873 (1)	0.104			
No	1.000	ref					
Yes	0.538	0.25 – 1.14					
eGFR			0.180 (1)	0.673			
Stage <3	1.000	ref					
Stage ≥3	0.864	0.44 – 1.70					
Insulin usage	1.378	0.68 – 2.80	0.813 (1)	0.376			
BMI, in kgm^{-2}			9.608 (1)	0.002	3.136	1.49 - 6.61	0.003
<30 kg/m^2	1.000	ref					
≥30 kg/m^2	2.865	1.49 – 5.50					
WC, in cm	1.055	1.02 – 1.09	12.194 (1)	0.001			
Central Obesity[#]			2.185 (1)	0.171			
No	1.000	ref					
Yes	2.145	0.72 – 6.39					
FBS, in mmol/L			1.621 (1)	0.209			
≤7.0	1.000	ref					
>7.0	1.493	0.80 – 2.79					
HbA1c, in %			3.041 (1)	0.118			
≤6.5	1.000	ref					
>6.5	2.674	0.78 – 9.18					
Platelet Count, in x 10^9/L			2.761 (1)	0.108			
<150	7.273	0.65 – 81.70					
≥150	1.000	ref					
Albumin in g/L			0.100 (1)	0.750			
<35	1.149	0.49 – 2.71					
35-52	1.000	ref					
ALT, in U/L[*]			14.774 (1)	<0.001			
Normal	1.000	ref					
Abnormal	3.244	1.75 – 6.00					
AST, in U/L			16.533 (1)	<0.001			
<32	1.000	ref					
≥32	4.398	2.18 – 8.87					
GGT, in U/L			47.000 (1)	<0.001	8.394	4.20 -16.78	<0.001
<40	1.000	ref					
≥40	8.883	4.56 – 17.29					
TC, in mmol/L			1.288 (1)	0.277			
<5.2	1.000	ref					
≥5.2	0.599	0.24 – 1.51					
TG, in mmol/L			6.633 (1)	0.010			
≤1.7	1.000	ref					
>1.7	2.219	1.21 – 4.06					
LDL in mmol/L[$]			0.822 (1)	0.376			
≤2.6	1.000	ref					
>2.6	0.700	0.32 – 1.54					
HDL in mmol/L[&]			6.275 (1)	0.013			
Normal	1.000	ref					
Abnormal	2.136	1.18 – 3.88					

Multiple logistic regression Forward LR method was used to identify significant variables
Multicollinearity and interaction were checked and not found.
Hosmer-Lemeshow test was not significant (p=0.967).
The Pseudo R2 was 0.286 and the model predicted 81.7% of cases correctly.
Missing values: LDL, 6.
[#] Waist circumference: Male ≥90 cm; Female ≥80 cm
* ALT: normal: <30 U/L Male; <19 U/L Female. Abnormal: ≥30 U/L Male; ≥19 U/L Female
[$] Missing values: LDL, 6. n=252
[&] HDL: normal: >1.0 mmol/L Male; >1.2 mmol/L Female. Abnormal ≤1.0 mmol/L Male; ≤1.2 mmol/L Female

According to the Malaysian National Health and Morbidity Survey in 2019, 19.7% of our adult population was obese.[31] Our cohort reflected that, with 20.9% of the patients being obese. In our study model, BMI and GGT were identified as two independent factors associated with advanced liver fibrosis. Reducing the high prevalence of obesity will reduce the prevalence of NAFLD and advanced liver fibrosis.

Based on the WHO expert consultation published in 2004, the recommended BMI cut-off for obesity was 30 kg/m^2. For many Asian populations, additional trigger points for public health action were identified with a BMI of at least 23 kg/m^2 representing increased risk and a BMI of at least 27.5 kg/m^2 representing high risk.[32] With this in mind, we categorised our cohort based on different BMI cut-offs. According to Figure 2, at different BMI cut-offs less than 23.0 kg/m^2, greater than or equal to 23.0 kg/m^2, greater than or equal to 27.5 kg/m^2 and greater than or equal to 30.0 kg/m^2, the prevalence of advanced liver fibrosis was 2.9%, 25.1%, 27.4% and 38.2% respectively. There was a marked increase of 10.8% prevalence between the cut-offs of 27.5 kg/m^2 and 30 kg/m^2. Therefore, identifying obesity at a lower cut-off of 27.5 kg/m^2 in our local population instead of 30 kg/m^2 will allow earlier public health and clinical intervention to reduce the prevalence of NAFLD and advanced liver fibrosis.

Currently, treatment approaches for patients with T2DM and NAFLD include weight loss with lifestyle modification, medications such as GLP-1 RA or SGLT2-inhibitors, bariatric surgery, optimising control of cardiovascular risks factors (i.e., T2DM, hypertension and dyslipidaemia) and liver-directed therapies such as pioglitazone. Pioglitazone has shown to improve liver histology in patients with and without T2D with biopsy-proven NASH.[33] These treatment modalities are needed to prevent or slow the progression of NAFLD to advanced fibrosis.

Liver biopsy is considered the reference standard but is impractical to apply to a large study population. With its known limitations, the development and application of new imaging modalities and diagnostic scores can reduce the need for liver biopsy. In 2012, FibroScan® with CAP measurement emerged as a novel non-invasive, easy-to-perform tool developed to assess both hepatic steatosis and fibrosis simultaneously with high sensitivity and specificity.[34] Screening for NAFLD using FibroScan® among individuals with T2DM was recommended in the 2017 Asia–Pacific Working Party on Non–Alcoholic Liver Disease guidelines.[35]

Our FibroScan® machine model could only measure LSM to diagnose fibrosis, but not CAP to assess liver fat. Diagnosing NAFLD early in the disease spectrum is important because early intervention especially with lifestyle modification and treatment of associated comorbidities already mentioned will slow NAFLD disease progression.

Fibrosis assessment is also clinically important. Fibrosis is the most powerful (and possibly the only independent) prognostic factor for liver-related outcomes in NAFLD, including hepatocellular carcinoma development and mortality.[36,37] Having any fibrosis, particularly significant fibrosis with a fibrosis score of at least F2, is associated with increased mortality.[38,39] Currently, there are six phase III trials investigating five agents (cenicriviroc, elafibranor, obeticholic acid, resmetirom and aramchol) that could potentially lead to histological resolution of NASH, no worsening of fibrosis, and even improvement of fibrosis score by at least 1 stage.[40-45]

Although a good modality for liver fibrosis screening, FibroScan® is not readily available in many health centres. Even if it is available, it is impossible to apply it universally to the large number of diabetic patients. In 2009, Shah et al., concluded that the FIB-4 index is superior to 7 other non-invasive markers of fibrosis in patients with NAFLD.[16]

In our study, using a cut-off of 1.31, the FIB-4 index showed a sensitivity, specificity, positive predictive value, and negative predictive value in detecting advanced liver fibrosis by LSM criteria of 30.0%, 85.0%, 38.7% and 79.4% respectively. With a high specificity and negative predictive value, the FIB-4 index can be used to exclude advanced liver fibrosis in centres where FibroScan® is not readily available. A prospective follow-up study of this cohort will be good to determine if the changes in FIB-4 scores correspond to changes in fibrosis over time.

Elevated serum ALT is often used as an indicator for further liver assessment in our clinical setting. However, it is important to note that serum ALT is not ideal for NAFLD screening as it may be normal across the spectrum of the disease,[46] and may even be normal or low in advanced liver fibrosis. Our study showed that ALT has low sensitivity and specificity in diagnosing advanced liver fibrosis.

It is important to note that the WHO guideline and Prati et al., defined the upper limit of normal ALT as 30 U/L for men and 19 U/L for women.[47,48] Usually, our local laboratories give a higher cut-off. For example, the laboratory in our study centre gives a single cut-off of 33 U/L. This would have misled many uninformed doctors and missed a large proportion of patients at risk of liver disease.

All available modalities have their strengths and limitationsand it is important to help decide when to use the appropriate test in the evaluation of patients with NAFLD and advanced liver fibrosis. It is important to identify the risk factors associated with advanced liver fibrosis to prioritise those at highest risk.

Our study had adequate sample size and power to examine the intended primary objective. We applied one of the best non-invasive tests for liver fibrosis to date. All FibroScan® examinations were performed by one dedicated

experienced operator to provide accurate and reliable LSM results. Lastly, we had comprehensive anthropometric and blood parameters for assessment.

Despite our best effort, there were nevertheless, several limitations. First, this was a single-centre study done in a tertiary care centre. This may not reflect the true prevalence in our population. Second, diagnosis of hepatitis B, C and autoimmune hepatitis were based on known medical history alone. Third, our FibroScan® machine model was only able to measure LSM to diagnose fibrosis, but not CAP to assess liver fat. Fourth, our centre did not have a dedicated ultrasonographer to perform hepatobiliary ultrasound to correlate with the FibroScan® findings. Lastly, our study did not include liver biopsy to assess the histological correlation with the LSM finding on FibroScan®.

CONCLUSION

Our study has confirmed the high prevalence of advanced liver fibrosis based on transient elastography among patients with long-standing T2DM. This study suggests the benefit of advanced liver fibrosis screening in patients with T2DM greater than 10 years in duration especially those with high BMI and GGT. Transient elastography and FIB-4 index have limitations as do other non-invasive tests for fibrosis. Understanding the caveats associated with the utility of each modality will optimize their use in clinical practice.

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

CRediT Author Statement
XHL: Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft preparation, writing – review and editing, visualization, supervision, project administration, funding acquisition; **LMN:** Formal analysis, investigation, resources, data curation, writing – review and editing, visualization; **CSA:** Formal analysis, investigation, resources, data curation, writing – review and editing, visualization; **TPY:** Conceptualization, methodology, validation, resources, writing – review and editing, visualization, supervision; **SLL:** Conceptualization, methodology, validation, writing – review and editing, visualization, supervision, project administration.

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ORIGINAL ARTICLE

The Role of Triglyceride-Glucose Index in the Prediction of the Development of Hypertension – Findings from a Community Cohort in Singapore

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Abstract

Objectives. Triglyceride-glucose index (TyGI) is an emerging surrogate marker of insulin resistance. We aim to explore the role of triglyceride-glucose index in the prediction of the development of hypertension.

Methodology. We conducted a retrospective cohort study that included 3,183 study participants identified from a community health screening programme who had no baseline hypertension and were then followed up after an average of 1.7 years. Cox proportional-hazard model was used to assess the association between risk of incident hypertension and TyGI in quartiles, while adjusting for demographics and clinical characteristics.

Results. Hypertension occurred in 363 study participants (11.4%). Those who developed hypertension had higher TyGI [8.6 (IQR 8.2-9.0)] than those who did not [8.2 (IQR 8.0-8.7)] (p<0.001). Significant association between TyGI and hypertension was observed in both the unadjusted and proportional hazard model [Quartile (Q)2, p=0.010; Q3, p<0.001 and Q4, p<0.001] and the model that adjusted for demographics (Q2, p=0.016; Q3, p=0.003; Q4, p<0.001). In the model adjusted for clinical covariates, the hazard of developing hypertension remained higher in TyGI Q4 compared to TyGI Q1(Hazard Ratio=2.57; 95% Confidence Interval: 1.71, 3.87). Increasing triglyceride-glucose index accounted for 16.4% of the association between increasing BMI and incident hypertension, after adjusting for age, gender, ethnicity and baseline HDL cholesterol (p<0.001).

Conclusion. Triglyceride-glucose index was an independent predictor of the development of hypertension. It may potentially be used as an inexpensive indicator to predict the development of hypertension and risk-stratify individuals to aid management in clinical practice.

Key words: type 2 diabetes mellitus, triglycerides, hypertension, screening, population science

INTRODUCTION

Hypertension poses a major risk for cardiovascular disease and mortality worldwide.[1-4] It was estimated that 31% of adults had hypertension globally in 2010.[5] As the population ages, the public health burden attributed to hypertension is expected to increase and the need to better understand and control the risk factors associated with the development of hypertension becomes more urgent.

One area of interest is the mounting evidence that shows hypertension and insulin resistance are linked.[4,6,7] However, it is challenging to use hyperinsulinemia-euglycemic clamp, the gold standard for assessing insulin resistance, in routine clinical practice as it is costly, time-consuming and often not readily available.[6,8] In recent years, triglyceride-glucose index (TyGI) has emerged as a promising surrogate of insulin resistance.[9,10] TyGI has been shown to correlate well with hyperinsulinemic-euglycemic clamp and homeostasis model assessment insulin resistance.[11-14]

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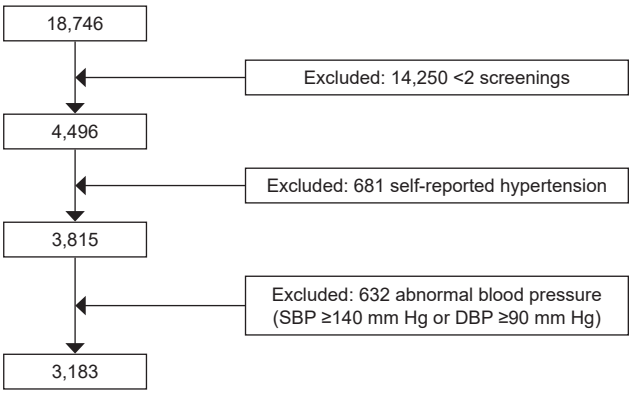
Furthermore, TyGI has been identified as an independent predictor of incident diabetes in a few studies.[8,10,15-17] Two studies in China and Spain also reported that TyGI conferred a higher risk of incident hypertension.[4,18] However, findings are still limited in view of the small number of studies. Interestingly, one study demonstrated interactions of TyGI and obesity on the risk of hypertension in a cross-sectional study.[6] To date, the mechanism on the role of TyGI in development of hypertension is still unclear. As such, we aimed to explore the role of TyGI in the development of hypertension and elucidate its role as a potential mediator in the association between body mass index (BMI) and the development of hypertension. We hypothesized that higher TyGI was linked to higher risk of hypertension, and that TyGI mediated the association between BMI and the development of hypertension.

METHODOLOGY

We conducted a retrospective cohort study on residents who attended the Alexandra Health Community Health Screening in the northern part of Singapore between September 2013 and December 2017. Of the 18,746 participants who were part of the health screening, 3,183 participants were included for analysis in this study (Figure 1). On average, the participants underwent 2-3 screenings with a follow-up period of 1.7 years. The study received ethics clearance from the National Healthcare Group Domain Specific Review Board in Singapore (Board (Ref. No. 2017/00735). Data was anonymized before analysis by the research team.

Information on demographics, smoking, exercise, stress coping strategies and medical history were obtained using a questionnaire administered to the participants as part of the screening. All readings were collected by community nurses and trained volunteers. Standing height and weight were obtained and body mass index (BMI) was calculated by dividing weight (in kg) by the square of height (in m^2). Sitting blood pressure after a resting period of at least 5 minutes was measured once with an automated sphygmomanometer (Omron, Japan) on the upper arm. Normal size soft cuffs (22-32 cm) were used except for individuals with obesity, for which the larger 32-42 cm soft cuffs were used. Morning fasting blood samples were collected from the participants, who had fasted for at least 9 hours overnight),and analysed at the hospital laboratory accredited by the Royal College of the American Pathologists. The following methods were used in the blood sample analysis: the hexominase method (Roche cobas® c701) for fasting plasma glucose (FPG), the enzymatic colorimeter test (Rocher cobas® c501) for high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG), and the Friedewald formula[19] for serum low-density lipoprotein cholesterol (LDL-C). The coefficients of variation (CV) for FPG were 0.5%-1.7% intra-assay and 0.4%-1.5% inter-assay. The CV for HDL-C were 0.4%-1.0% intra-assay and 0.9%-1.5% inter-assay, and the CV for TG were 0.7%-1.1% intra-assay and 1.6%-2.0% inter-assay.[20-22]



SBP, systolic blood pressure; DBP, diastolic blood pressure

Figure 1. Study population and exclusion criteria.

Exposure definition

The exposure variable, TyGI, was calculated using the formula by Simental-Mendia LE, et al.[12] : TyGI = Ln [fasting TG level (mg/dl)] x FPG (mg/dl)/2]. As there are no defined cut-offs for TyGI, participants were divided into quartiles according to their TyGI levels as follows: quartile (Q)1: 7.7-8.0); Q2: 8.1-8.3; Q3: 8.5-8.6; and Q4: 8.9-9.3.

Outcome definition

The outcome was the presence of hypertension defined as one of the following: systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, self-reported hypertension, or use of anti-hypertensive medications according to the World Health Organisation criteria.[23]

Statistical analysis

Categorical variables were expressed as frequencies (percentages). Continuous variables were expressed as mean (standard deviation) or median (interquartile range) depending on the distribution of variables. Additionally, TyGI was categorized into quartiles.

One-way analysis of variance and Student's t-test were used to compare the means of continuous variables across TyGI quartiles and hypertension status, respectively. Kruskal Wallis test and Mann-Whitney U test were used in lieu of these tests when the continuous variable had non-normal distribution. Chi-square test was used to compare the proportions of categorical variables across these groups.

Kaplan-Meier survival curves for incident hypertension stratified by TyGI quartiles were produced. These survival curves were compared using the log-rank test. On the other hand, Cox proportional-hazard regression was used to determine the association between TyGI and hypertension while controlling for age, gender, ethnicity and clinical covariates. Schoenfeld residuals were used to check if the proportional hazards assumption was violated.

Table 1. Baseline characteristics of participants stratified by triglyceride-glucose index (TyGI) in quartiles

Variable	All	TyGI quartile				
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	*p*
N	3183	805	793	790	795	
Age (years)	55.9 ± 8.1	54.7 ± 8.0	55.6 ± 8.0	56.6 ± 8.1	56.7 ± 8.3	<0.001
Male (%)	1035 (32.5)	155 (19.3)	209 (26.4)	288 (36.5)	383 (48.2)	<0.001
Ethnicity (%)						<0.001
Chinese	2776 (87.2)	715 (2.7)	724 (91.3)	682 (86.3)	655 (82.4)	
Malay	189 (5.9)	47 (5.8)	31 (3.9)	46 (5.8)	65 (8.2)	
Indian	144 (4.5)	21 (2.6)	24 (3.0)	45 (5.7)	54 (6.8)	
Other	74 (2.3)	22 (2.7)	14 (1.8)	17 (2.2)	21 (2.6)	
Exercise per week (%)						<0.001
120-150 mins	876 (27.5)	238 (29.6)	229 (28.9)	236 (29.9)	173 (21.8)	
60-90 mins	687 (21.6)	189 (23.5)	187 (23.6)	152 (19.2)	159 (20.0)	
30 mins	460 (14.5)	94 (11.7)	118 (14.9)	123 (15.6)	125 (15.7)	
<30 mins	1160 (36.4)	284 (35.3)	259 (32.7)	279 (35.3)	338 (42.5)	
Smoking (%)						<0.001
No	2970 (93.3)	772 (95.9)	753 (95.0)	730 (92.4)	715 (89.9)	
Yes	213 (6.7)	33 (4.1)	40 (5.0)	60 (7.6)	80 (10.1)	
Coping well with stress (%)						0.464
No	2174 (68.3)	551 (68.5)	558 (70.4)	528 (66.8)	537 (67.6)	
Yes	1009 (31.7)	254 (31.6)	235 (29.6)	262 (33.2)	258 (32.5)	
BMI (kg/m^2)	23.0 ± 3.6	21.4 ± 3.0	22.4 ± 3.4	23.6 ± 3.6	24.8 ± 3.4	<0.001
WC (cm)	81.6 ± 9.8	76.1 ± 8.4	79.7 ± 8.8	83.5 ± 9.3	87.3 ± 8.9	<0.001
SBP (mmHg)	120.1 ± 11.5	116.4 ± 11.9	119.6 ± 11.6	121.6 ± 10.8	123.0 ± 10.8	<0.001
DBP (mmHg)	72.1 ± 8.2	69.1 ± 7.9	71.5 ± 8.3	73.3 ± 7.8	74.5 ± 7.6	<0.001
LDL-C (mmol/l)	3.3 ± 0.9	2.9 ± 0.7	3.2 ± 0.8	3.5 ± 0.8	3.5 ± 0.9	<0.001
HDL-C (mmol/l)	1.6 ± 0.4	1.9 ± 0.4	1.7 ± 0.4	1.5 ± 0.4	1.3 ± 0.3	<0.001
TG (mmol/l)	1.0 (0.8-1.5)	0.6 (0.6-0.7)	0.9 (0.8-1.0)	1.2 (1.1-1.4)	1.9 (1.6-2.4)	<0.001
FPG (mmol/l)	5.3 ± 1.0	5.0 ± 0.4	5.2 ± 0.5	5.3 ± 0.6	5.9 ± 1.7	<0.001
TyGI	8.4 (8.1-8.8)	7.8 (7.7-8.0)	8.2 (8.1-8.3)	8.5 (8.5-8.6)	9.0 (8.9-9.3)	<0.001

TyGI, triglyceride-glucose index; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; FPG, fasting plasma glucose

Values presented as mean ± SD, frequencies (percentages), and median (IQR)

The role of TyGI on the association between BMI at baseline and the development of hypertension was assessed with mediation analysis. According to the Baron and Kenny framework,[24] mediation occurred if there were significant association between exposure and potential mediator, significant association between exposure and outcome and the association between exposure and outcome was attenuated when the potential mediator was included in the model. The two-sided tests performed were considered statistically significant if $p<0.05$. Analysis was done using STATA Version 14.0 (STATA Corp., College Station, TX, USA).

RESULTS

Table 1 shows the baseline characteristics of the study participants: age ($p<0.001$), BMI ($p<0.001$), SBP ($p<0.001$), DBP ($p<0.001$), LDL-C (p<0.001), TG ($p<0.001$) increased across TyGI quartiles, whereas HDL-C ($p<0.001$) decreased across TyGI quartiles. Study participants with higher TyGI quartiles tended to be males and non-Chinese ($p<0.001$). Those with higher TyGI quartiles were more likely to smoke and exercise less (≤30 mins per week) ($p<0.001$). Additionally of note, the overall mean HDL-C level was high and median TG level was low, potentially attributable to lower overall smoking rates (6.7%) and higher proportions who exercise at least 30 mins a week (63.6%).

After 5,380.07 person-years of follow-up, 363 study participants developed hypertension (11.4%) (Table 2). These study participants tended to be males and had poorer clinical profiles in terms of BMI, SBP, DBP, LDL-C, HDL-C, FPG and TyGI ($p<0.05$).

The survival curves for incident hypertension stratified by TyGI quartiles are shown in Figure 2. Results of the

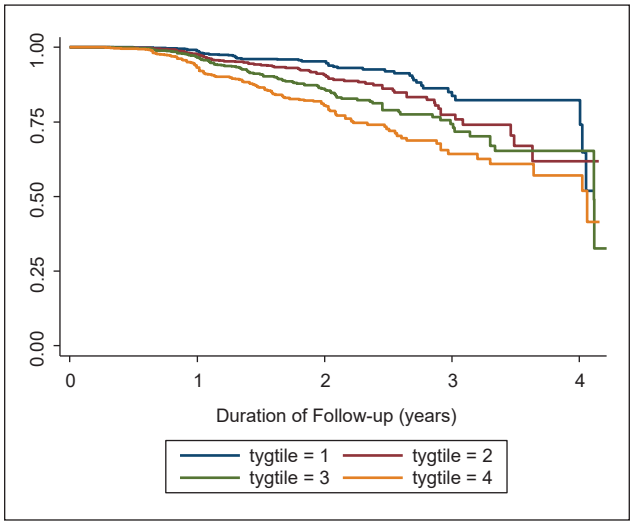


Figure 2. Kaplan-Meier survival curves by triglyceride-glucose quartiles.

log-rank test indicated that these survival curves were significantly different (p<0.001) with TyGI Q3 and Q4 having poorer disease-free survival compared to TyGI Q1 and Q2.

Table 2. Baseline characteristics of participants stratified according to the development of hypertension

Variable	Development of hypertension		
	No	Yes	*p*
N	2820	363	
Age (years)	55.4 ± 8.0	59.7 ± 8.3	<0.001
Male (%)	884 (31.4)	151 (41.6)	<0.001
Ethnicity (%)			0.816
Chinese	2462 (87.3)	314 (86.5)	
Malay	168 (6.0)	21 (5.8)	
Indian	127 (4.5)	17 (4.7)	
Other	63 (2.2)	11 (3.0)	
Exercise per week (%)			0.291
120-150 mins	775 (27.5)	101 (27.8)	
60-90 mins	613 (21.7)	74 (20.4)	
30 mins	396 (14.0)	64 (17.6)	
<30 mins	1036 (36.7)	124 (34.2)	
Smoking (%)			0.948
No	2631 (93.3)	339 (93.4)	
Yes	189 (6.7)	24 (6.6)	
Coping well with stress (%)			0.234
No	1936 (68.7)	238 (65.6)	
Yes	884 (31.4)	125 (34.4)	
BMI (kg/m^2)	22.9 ± 3.5	24.1 ± 3.9	<0.001
WC (cm)	81.2 ± 9.7	85.2 ± 10.1	<0.001
SBP (mmHg)	118.9 ± 11.3	129.9 ± 7.7	<0.001
DBP (mmHg)	71.3 ± 8.0	77.9 ± 7.1	<0.001
LDL-C (mmol/l)	3.3 ± 0.8	3.4 ± 0.9	0.002
HDL-C (mmol/l)	1.6 ± 0.4	1.5 ± 0.4	<0.001
TG (mmol/l)	1.0 (0.8-1.4)	1.2 (0.9-1.7)	<0.001
FPG (mmol/l)	5.3 ± 1.0	5.6 ± 1.3	<0.001
TyGI	8.3 (8.0-8.7)	8.6 (8.2-9.0)	<0.001

TyGI, triglyceride-glucose index; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; FPG, fasting plasma glucose

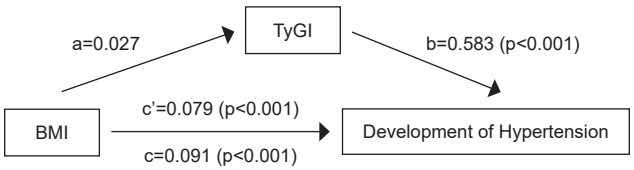
Values presented as mean ± SD, frequencies (percentages), and median (IQR)

The unadjusted Cox proportional hazard model (Table 3) showed that the hazard of hypertension increases with increasing TyGI. This hazard triples when the TyGI Q1 group is compared with the TyGI Q4 group (hazard ratio [HR]=3.31, 95% Confidence Interval [CI]: 2.38, 4.60). This association between higher TyGI quartiles and the development of hypertension remained statistically significant even after adjusting for demographics (Model 1) and baseline clinical covariates (Model 2), with the hazard for TyGI Q4 being more than double that of the TyGI Q1 (HR 2.57 (95%CI: 1.71, 3.87).

Mediation analysis (Figure 3) showed that BMI was associated with TyGI and the development of hypertension. Furthermore, there was attenuation of the relationship between BMI and development of hypertension with inclusion of TyGI in the model. After adjusting for age, gender, ethnicity and HDL-C, TyGI accounted for 16.4% of the relationship between BMI and development of hypertension (p<0.001).

DISCUSSION

Our findings revealed that higher TyGI was significantly associated with the development of hypertension. This was in line with the results from earlier research which showed that TyGI was a predictor of incident hypertension.[4,18]



Adjusted for age, gender, ethnicity and HDL-cholesterol.

Figure 3. Mediation of TyGI between baseline body mass index and the development of hypertension.

Table 3. Association between triglyceride-glucose (TyG) index in quartiles and the development of hypertension

Variable	Hazards Ratio (95% Confidence Interval)					
	Unadjusted	*p*	Model 1	*p*	Model 2	*p*
Age (per year)	1.06 (1.05, 1.08)	<0.001	1.06 (1.05, 1.07)	<0.001	1.06 (1.05, 1.08)	<0.001
Male	1.05 (1.22, 1.86)	<0.001	1.24 (1.00, 1.53)	0.046	1.34 (1.07, 1.68)	0.012
Ethnicity						
Chinese	0.92 (0.51, 1.68)	0.791	0.75 (0.41, 1.38)	0.355	0.85 (0.46, 1.56)	0.593
Malay	1.01 (0.49, 2.09)	0.982	0.85 (0.41, 1.77)	0.670	0.76 (0.36, 1.58)	0.460
Indian	1.03 (0.48, 2.19)	0.947	0.81 (0.38, 1.73)	0.585	0.81 (0.37, 1.74)	0.581
Other	1.00		1.00		1.00	
BMI (per kg/m^2)	1.08 (1.05, 1.11)	<0.001			1.08 (1.05, 1.11)	<0.001
LDL-C (per mmol/l)	1.20 (1.06, 1.35)	0.003			1.04 (0.91, 1.17)	0.577
HDL-C (per mmol/l)	0.67 (0.53, 0.86)	0.001			1.29 (0.93, 1.78)	0.126
TyGI						
Quartile 1	1.00		1.00		1.00	
Quartile 2	1.61 (1.12, 2.32)	0.010	1.56 (1.09, 2.25)	0.016	1.50 (1.03, 2.18)	0.035
Quartile 3	2.23 (1.58, 3.14)	<0.001	1.97 (1.40, 2.78)	<0.001	1.78 (1.22, 2.62)	0.003
Quartile 4	3.31 (2.38, 4.60)	<0.001	2.89 (2.07, 4.03)	<0.001	2.57 (1.71, 3.87)	<0.001

TyGI, triglyceride-glucose index; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol
Model 1 adjusted for age, gender, and ethnicity. Model 2 adjusted for age, gender, ethnicity, BMI, LDL-C, and HDL-C.

While the underlying mechanism for the relationship was not clear, there are a few possible explanations for insulin resistance conferring higher risk for the development of hypertension. First, insulin resistance enhances the activity of the sympathetic nervous system and increases the release of catecholamines, thereby causing thickening of the vascular smooth muscle and luminal stenosis.[25-28] The second possible explanation is that insulin resistance may lead to production and release of endothelin, narrowing blood vessels with less prostaglandin E2 and prostacyclin generated. This would then cause an increase in smooth muscle vasculature which would then elevate the blood pressure.[4,29,30] Third, insulin resistance enhances renin-angiotensin-aldosterone system activity and contributes to sodium reabsorption in the proximal tubule.[6,31-33] As TyGI was considered a surrogate of insulin resistance[9,10] and was also correlated positively with waist circumference (a marker of insulin resistance) in our study (correlational coefficient 0.457; $p<0.001$), it is then plausible that the above mechanisms accounted for the relationship between TyGI and the development of hypertension.

TyGI was observed to mediate the relationship between BMI and the development of hypertension in our study, suggesting partial contribution by TyGI, which is an indicator of insulin resistance, to the detrimental impact of BMI on the development of hypertension. Interestingly, an earlier study reported an interaction between TyGI and obesity on the risk of hypertension in middle-aged and elderly adults.[6] In our earlier study, TyGI was shown to mediate the association between BMI and the development of diabetes.[17] Thus it is plausible that TyGI partly accounted for the deleterious effects of BMI on cardiovascular and metabolic risk.

Our study has several strengths. First, this was a large community cohort. Second, mediation analysis was done to enhance our understanding of the role of the TyGI on the development of hypertension. However, because the large community cohort was used, we were limited by a relatively short follow-up period, retrospective design, and lack of data on insulin levels to confirm the mechanism underlying the association between insulin resistance and hypertension. Blood pressure was only measured once and a single reading could be misleading as BP is affected by transient external events. We also lacked information on alcohol intake which could potentially be a cofounding factor. Furthermore, while encouraged, repeat screenings were fully voluntary. This potentially led to some level of self-selection, with more motivated participants possibly following up more frequently.

TyGI is an inexpensive measure which is easily available in routine clinical practice. It may potentially be utilized to identify individuals at higher risk for hypertension, thereby enabling healthcare providers to form a stratified approach in the management through more targeted lifestyle interventions and medications. The mediating effect of TyG on the association between BMI and the development of hypertension also highlights the clinical importance of promoting a healthy body weight in an effort to reduce the risk of hypertension. Moving forward, it would be worth returning to this cohort after a longer time period to further explore the development of hypertension and other outcomes such as stroke, heart attack, and mortality. Our methodology is easily repeatable with other large health screening cohorts that can corroborate our observations.

CONCLUSION

In conclusion, triglyceride-glucose index was found to be an independent predictor of the development of hypertension and may potentially be used as an inexpensive indicator to predict the development of hypertension and risk-stratify individuals to aid management in clinical practice.

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

CRediT Author Statement
JKCK: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing – original draft, Writing – review and editing, Visualization, Project Administration, Funding Acquisition; **SL:** Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – original draft, Writing – review and editing, Visualization, Project Administration Funding Acquisition; **BI:** Conceptualization, Investigation, Resources, Writing – review and editing; **JIST:** Conceptualization, Validation, Writing – review and editing; **CFS:** Conceptualization, Writing – review and editing, Supervision; **TS:** Conceptualization, Writing – review and editing, Supervision; **SCL:** Conceptualization, Methodology, Writing – review and editing, Supervision

Author Disclosure
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ORIGINAL ARTICLE

Clinicodemographic Profile and Outcomes of Type 2 Diabetes Mellitus in the Indonesian Cohort of DISCOVER: A 3-Year Prospective Cohort Study

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Abstract

Background. Indonesia is amongst the top 10 countries with the highest prevalence of Type 2 Diabetes Mellitus (T2DM) at 10.8%. However, the distinguishable features of T2DM in Indonesia remain obscure. Therefore, the DISCOVER study aimed to describe the characteristics of T2DM patients, associated vascular complications and treatment in Indonesia.

Methodology. DISCOVER study is a multi-country, multicenter, prospective, cohort study over 3 years. In the present study, the data were collected from 13 sites from clinical practice, hospitals and public health facilities in Indonesia.

Results. A total of 221 subjects were recruited with a mean age of 55.6 ± 9.8 years and body mass index (BMI) of 26.4 ± 4.4 kg/m^2. Over 40% of patients had hypertension and/or hyperlipidemia. The mean duration of T2DM was 58.3 ± 62.0 months while the mean HbA1c levels was 9.2 ± 2%. In total, 82.4% completed the study within a 36-month follow-up period. BMI remained elevated i.e., >25 kg/m^2. A significant reduction was observed in HbA1c levels as compared to baseline (9.2 ± 2% to 8.1 ± 1.8%). T2DM-associated microvascular complications such as peripheral neuropathy, albuminuria and chronic kidney disease were observed in 17.2%. Macrovascular complications including coronary artery disease and heart failure were seen in 26.2% of patients. We also found that more than 70% of patients were on metformin and/or sulfonylurea.

Conclusion.The features of patients with T2DM in Indonesia were high BMI, with hypertension and hyperlipidemia as co-morbidities. Metformin and sulfonylureas were the most common treatment. HbA1c reduction during follow-up did not reach recommended target. Thus, early detection and intervention using available glucose-lowering medications and aggressive management of risk factors and complications are essential to improve outcomes of diabetes management in Indonesia.

Key words: diabetes type 2, vascular complications, real-world study, Indonesia

INTRODUCTION

Type 2 diabetes mellitus (T2DM) affects an estimated 463 million adults aged 20–79 years and is projected to reach 578 million people in 2030. It imposes a significant economic burden on the global healthcare system and the broader global economy. In 2016-2017, the average cost for T2DM outpatients based on National Health Coverage claims was USD 9574 per 7 days of treatment.[1]

There is limited published data on the incidence and prevalence of T2DM in Indonesia. According to the International Diabetes Federation (IDF), Indonesia's national diabetes prevalence is estimated to be 6.2% in 2019 and 10.8% in 2021, placing it among the top 10 countries with the highest prevalence of T2DM and also with the steepest climb.[1]

According to the Basic Health Research (*Riset Kesehatan Dasar*/ RISKESDAS) 2018 in Indonesia, 10.9% of population ≥15 years old have T2DM. Majority of these patients also have acute or chronic complications.[2,3] Hyperglycemia is associated with several potentially life-threatening microvascular and macrovascular complications, including

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heart failure, coronary artery disease (CAD) and chronic kidney disease (CKD).[3] Due to these complications, diabetes poses risks of decreased quality of life and high economic burden, making it a critical chronic disease to address.

Clinical guidelines recommend metformin as the first line glucose-lowering therapy, in conjunction with lifestyle changes. The Indonesian Endocrinologist Society, *Perkumpulan Endokrinologi Indonesia* (PERKENI), recommends several glucose-lowering therapies as the first-line according to the patient's glycated hemoglobin (HbA1c) in conjunction with lifestyle changes.[3] Sustaining glycemic control, in conjunction with the management of comorbidities such as hypertension and hyperlipidemia, continues to be a critical component of effective T2DM treatment. If metformin monotherapy fails, however, guidelines recommend an individualized and patient-centered approach to drug selection based on patient characteristics.[4]

DISCOVER study's primary objective is to characterize the disease management patterns and clinical evolution of T2DM patients initiating a second-line glucose-lowering treatment (add-on or switch) over 3 years. The purpose of this research was to describe baseline data and its changes in 3 years of follow-up on T2DM patients in Indonesia and to discuss the prevalence of cardiorenal complications following the initiation of second-line therapy.

METHODS

DISCOVER is a 3-year multi-country, multi-center, prospective, cohort study. The study was approved by each participating institution's Institutional Review Board (IRB). A signed informed consent form was obtained from each participant.

For the Indonesian cohort, a total of 221 patients were recruited randomly from 13 sites throughout the country's 8 provinces from January 2015 - October 2019. These sites were selected on the basis of the proportion of patients in primary, secondary, tertiary, private or state owned health care facilities. The study population consisted of patients aged 18 years and above with a diagnosis of T2DM, and who were initiating a second-line glucose-lowering therapy. Patients with previous T2DM diagnosis were also included if they had micro- or macrovascular complications. Macrovascular complications include heart failure, coronary artery disease, and diabetic foot while microvascular complications include chronic kidney disease and peripheral neuropathy events. Excluded in the study were patients who were pregnant, those undergoing dialysis, had renal transplant and those who received injectable agents as first-line therapy or traditional regimen.

Medical records were reviewed for patient demographics, clinical characteristics and glucose-lowering treatment. Laboratory results, as well as history of complications or related procedures were noted during the initial clinical visit and subsequent regular clinical visits at 6, 12, 24 and 36 months. Complications were diagnosed and classified by the investigators who were practicing physicians at the respective study centress and were validated by 2 different investigators as per the patient's medical record.

The demographic variables such as patient's baseline characteristics, treatment patterns, HbA1c level, fasting blood glucose (FBG) and postprandial glucose (PPG), lipid profile, body weight, body mass index (BMI), blood pressure, cardiorenal outcomes and hospitalization events were described using descriptive statistics. As appropriate, descriptive data were presented as frequencies (percentages), means (standard deviations [SD]) and medians (interquartile ranges [IQR]). The data were analyzed using Graphpad v5 software. The baseline and follow-up data were compared statistically using repeated measure ANOVA.

For sample size calculation, statistical software, 'G*Power version 3.1.9.2' was used taking the prevalence of T2DM in the Indonesian population to be 10.8%. The sample size also considered other factors such as follow-up time of 36 months, complications and mortality rate. Considering all these factors and keeping 80% power and 5% significance, the sample size was estimated to be 285.

RESULTS

The Indonesia DISCOVER study program enrolled a total of 221 patients from 13 sites in Indonesia, including primary care centers (8.3%), general/community hospital (50%), and university/teaching hospital (25%). The clinical sites were Endocrinology (38.5%), Internal Medicine (30.8%) and General Practitioner practices (20.8%). Public hospital/ health center accounted for 61.5% of cases. Of 221 patients at baseline, 182 (82.4%) completed the study during the 36 months follow-up period, 5.9% of patients died and 8.6% were lost to follow-up. Cardiovascular disease accounts for 50% of these deaths. Reasons for discontinuation in the study include withdrawal of consent (2.3%) and other reasons (0.9%). Table 1 shows the baseline demographics i.e., age, gender, ethnicity, working status, medical history, health insurance coverage and diabetes-related complications. All participants were Indonesians (Asian) with a mean age of 55.6 ± 9.8 years. Among them, 56.6% were females. Over 40% of patients had comorbidities such as hypertension and/or hyperlipidemia.

The initial BMI was 26.4 ± 4.4 kg/m^2 and remained elevated throughout the follow-up period, i.e., >25 kg/m^2. The mean duration of T2DM was 58.3 ± 62.0 months with HbA1c levels of 9.2 ± 2% at baseline. There was a significant reduction in HbA1c, FBG and PPG during follow-up as compared to baseline (p<0.05). However, the mean HbA1c level (8.0 ± 1.8) did not reach the American Diabetes Association (ADA) recommended target of 7%.[3] Unfortunately, more than 40% of data were missing for HbA1c and PPG. Other clinical characteristics such as systolic and diastolic

Table 1. Patient demographics

Parameter, n (%)	n = 221
Sex	
Male	96 (43.4%)
Female	125 (56.6%)
Age, years (Mean±SD)	55.6 ± 9.8
Self-reported ethnicity	
Caucasian	1 (0.5%)
Asian	205 (92.8%)
Arabic	2 (0.9%)
Others	13 (5.9%)
Education level	
No formal education	4 (1.8%)
Primary (1-6 years of education)	41 (18.7%)
Secondary (7-13 years of education)	72 (32.9%)
University/higher education (13+ years)	102 (46.6%)
Not known	2 (0.9%)
Main working status	
Employed	70 (31.8%)
Self-employed	47 (21.4%)
Unemployed	82 (37.3%)
Retired	21 (9.5%)
Not known	1 (0.45%)
Health insurance coverage	
Private	61 (27.6%)
Public/governmental	105 (47.5%)
Mixed	1 (0.5%)
No insurance	54 (24.4%)
Duration of diabetes (months), mean±SD	58.3 ± 62.0
Comorbidities	
Hypertension	97 (43.9%)
Hyperlipidemia	98 (44.3%)
Macrovascular complication	38 (17.2%)
Heart failure	11 (5%)
Coronary artery disease	12 (5.4%)
Diabetic foot	9 (4.1%)
Microvascular complication	58 (26.2%)
Chronic kidney disease	14 (16.2%)
Peripheral neuropathy event	20 (9%)

SD, standard deviation; n, number of patients; %, percentage

blood pressure, weight and lipid profile (total cholesterol, high-density lipoproteins, and low-density lipoproteins) remained the same during follow-up. On the contrary, triglycerides levels were significantly reduced. Table 2 summarizes the clinical characteristics at baseline and follow-up.

Microvascular and macrovascular complications associated with T2DM were observed in 17.2% and 26.2% of patients, respectively. As shown in Figure 1 (A and B), the most common macrovascular complications at 36 months were coronary artery disease and heart failure in 35% and 25%, respectively. On the other hand, peripheral neuropathy contributed to 32% of cases, microalbuminuria in 25% of cases and chronic kidney disease in 20% of cases with microvascular complications. Diabetes-related complications at follow-up were further documented and classified based on the need for hospitalization. There were 6.2% hospitalization events in 6 months, 5.6% in 12 months and 6.4% in 24 months follow-up. A surge of 12.1% of hospitalization events was observed at 36 months of follow-up. The primary reasons for hospitalization were cardiovascular events, diabetic foot and diabetes-related complications, which are shown in Figure 1 (C). Serious infections, cancer, cellulitis and renal failure were included in other reasons.

In terms of medications, approximately 36.7% of patients were started on monotherapy and 13.6% of patients were on combination therapy. We found that more than 70% of patients were on metformin and/or sulfonylurea as a first-line treatment. The combination of metformin and sulfonylurea was the most frequently used second-line therapy, followed by the triple therapy of metformin, sulfonylurea and dipeptidyl peptidase-4 inhibitor (DPP-4i). About 26.7% of patients were on second-line therapy and 11.3% were on triple therapy. During the follow-up period, only 22.7% of patients remained on monotherapy. The most frequently cited reason for switching from first-line to second-line therapy was due to treatment failure. The choice for second-line therapy was based on improved efficacy. Sulfonylureas were the most commonly stopped medication at all follow-up time points, accounting for 6.7%, 8.7%, 8% and 8.4% of the population at 6, 12, 24, and 36 months, respectively, followed by metformin and fixed-dose metformin+DPP-4i. The changes in patients' treatment during the follow-up period of 36 months are shown in Figure 2.

Table 2. Clinical characteristics at baseline and follow-up time points (6, 12, 24, 36 months)

Parameter	Baseline data (0 months) n = 221	6 months follow-up n = 195	12 months follow-up n = 196	24 months follow-up n = 188	36 months follow-up n = 190	*p*
Weight (kg)	67.4 ± 14.0	67.0 ± 14.1	67.0 ± 14.1	67.2 ± 14.1	67.4 ± 14.5	1.000
BMI (kg/m^2)	26.4 ± 4.4	26.3 ± 4.4	26.3 ± 4.4	26.4 ± 4.5	26.4 ± 4.6	1.000
Systolic BP (mmHg)	127.7 ± 17.2	128.9 ± 17.6	128.4 ± 16.4	129.3 ± 16.9	129.7 ± 19.5	0.278
Diastolic BP (mmHg)	80.6 ± 8.7	80.5 ± 7.8	80.6 ± 7.6	81.2 ± 8.7	81.0 ± 10.0	0.671
HbA1c (%)	9.2 ± 2	7.9 ± 1.7	7.9 ± 1.8	8.1 ± 1.9	8.0 ± 1.8	**<0.01**
Fasting glucose (mg/dL)	176.5 ± 60.3	150.2 ± 52.7	155.4 ± 55.0	162.5 ± 60.2	156.1 ± 51.3	**<0.05**
PPG (mg/dL)	250.0 ± 81.9	208.4 ± 69	212.3 ± 75.4	212.3 ± 75.4	221.1 ± 78.1	**<0.01**
HDL-C (mg/dL)	46.3 ± 12.7	45.6 ± 10.1	48.2 ± 12.3	47.1 ± 12.9	48.7 ± 13.3	0.063
LDL-C (mg/dL)	126.8 ± 39.1	123.2 ± 37.3	124.7 ± 34.6	126.5 ± 39.0	127.7 ± 39.2	0.834
Total cholesterol (mg/dL)	204.9 ± 54.5	196.2 ± 43.0	206.1 ± 50.5	201.0 ± 50.5	194.7 ± 42.2	0.052
Triglycerides (mg/dL)	186.4 ± 182.5	155.8 ± 74.5	177.1 ± 194.2	160.7 ± 81.6	154.7 ± 109.7	**<0.05**

BMI, body mass index; BP, blood pressure; PPG, postprandial glucose; SD, standard deviation; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

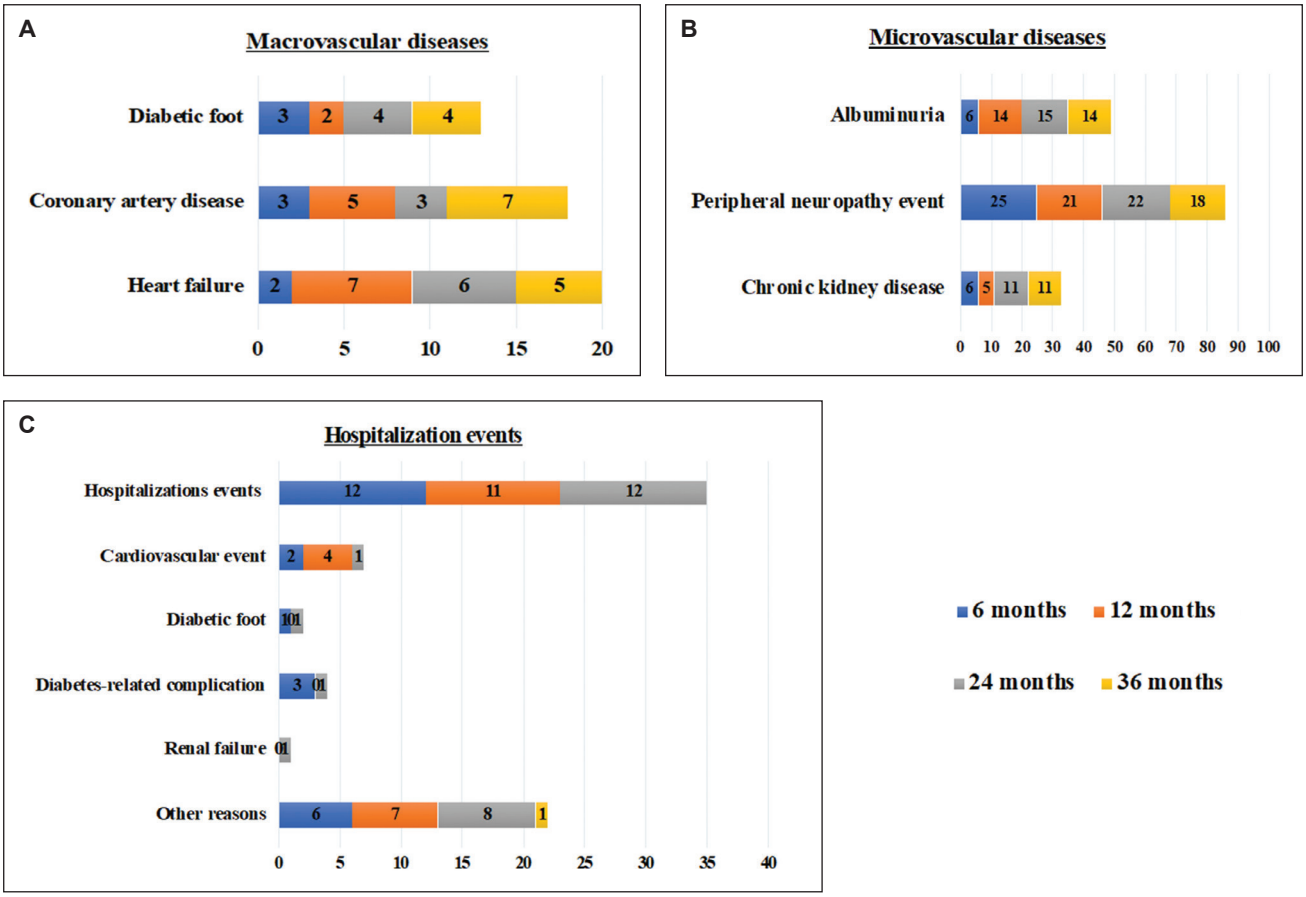

Figure 1. Diabetes-related complications during a follow-up period of 36 months. Side bar-graphs depict the frequencies of participants affected with diabetes-related complications including **(A)** Macrovascular diseases, **(B)** Microvascular diseases, and **(C)** hospitalization events during a follow-up period of 36 months.

DISCUSSION

DISCOVER is a global research programme that assessed the characteristics, treatment and outcomes of T2DM patients after initiating second-line glucose-lowering therapy. This is the first of Indonesia's DISCOVER study from 13 clinical sites representing primary and secondary care in rural and urban locations with different treatment cost resources. This observational study gives a broad picture of the real-world management of patients with T2DM in Indonesia, with different backgrounds, ethnicities and socioeconomic status.

Compared to the whole DISCOVER study, the patients in the Indonesian cohort were younger (55.6 ± 9.8 vs 57.5 ± 12), majority were female (566 vs 47.3%), with lower BMI (26.4 ± 4.4 vs 29.4 ± 6) but higher HbA1c (9.2 ± 2 vs 8.4 ± 1.7).[5] The mean BMI of patients w 26.4±4.4 kg/m^2 which can be classified as overweight or obese. In a study by Cholil et al., a similar mean BMI (25.4 ± 4.2 kg/m^2) was observed among T2DM patients in Indonesia.[6]

Several studies have shown the presence of other medical conditions such as hypertension and dyslipidemia among patients with T2DM.[6-8] The present study demonstrated that T2DM patients in Indonesia had systolic blood pressure of 127.7 ± 17.2 mmHg and diastolic blood pressure of 80.6 ± 8.7 mmHg, which can be classified as pre-hypertension and were stable over 3 years. Additionally, we observed that the mean lipid parameters were elevated over a three-year follow-up period, indicating that most patients had dyslipidemia.[4,5,9]

Monitoring the HbA1c level is recommended to aid in treatment decisions in T2DM patients. The risk for T2DM complications increases linearly with HbA1c levels making glycemic control critical.[10] Some studies indicate that aggressively lowering HbA1c levels increases the rate of hypoglycemia, possibly increasing the risk of cardiovascular events. Compliance with HbA1c testing was low in Indonesia due to the price and unavailability in some provinces. After 3 years of follow-up, mean HbA1c levels decreased significantly but did not reach the target (8.0 ± 1.8%). In several DISCOVER studies, the patients who were started on second-line therapies had a mean HbA1c level that was also above target and remained consistently high even after changing therapies.[5,10,11] The study population's glycemic control was unsatisfactory based on Diabcare 2008 and 2012. Only about one-third of patients met the ADA's recommended HbA1c and FPG targets and were above the recommended PPG levels.[6,12] This indicates that a sizable proportion of patients had suboptimal glycemic control and delayed treatment intensification, increasing their risk of microvascular and macrovascular complications.[13]

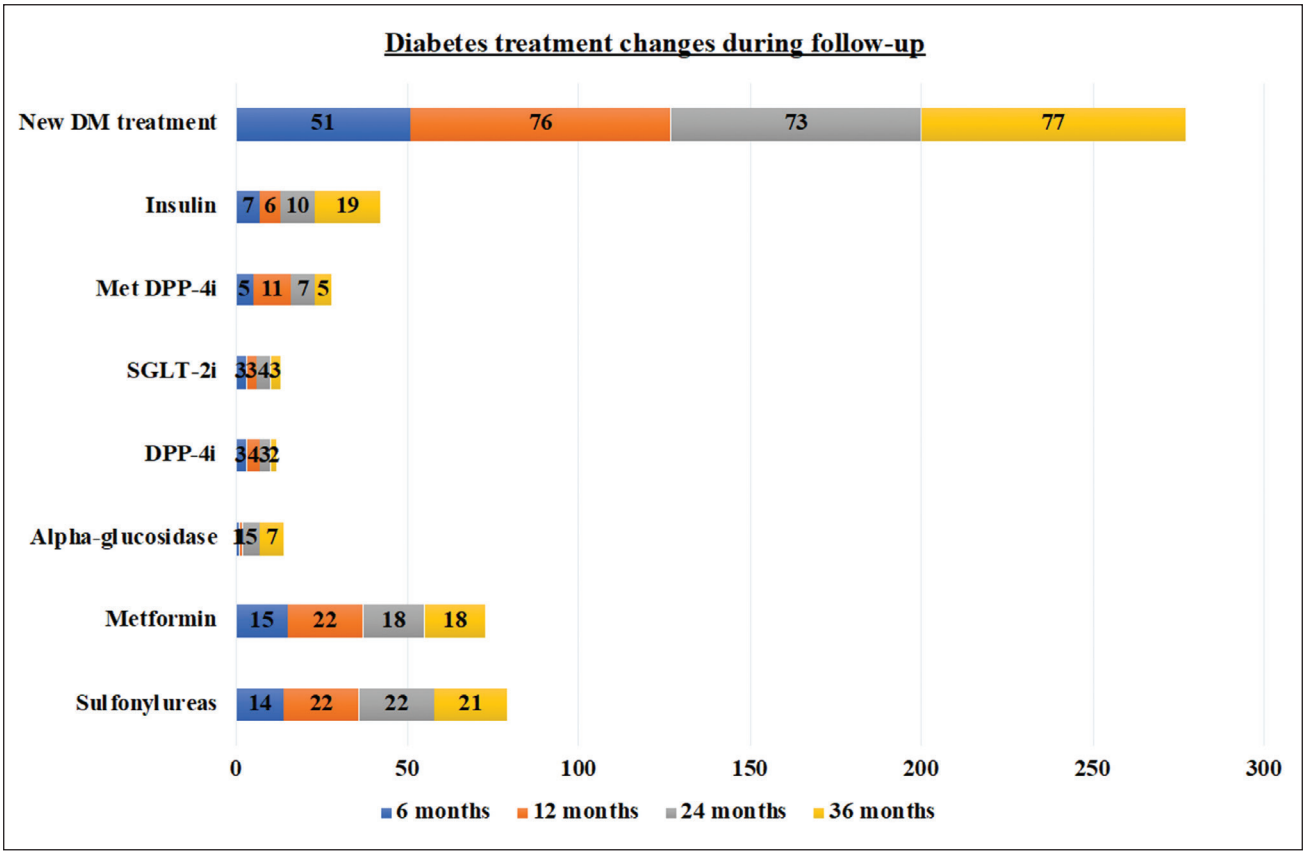

Figure 2. Changes in diabetes treatment during a follow-up period of 36 months. Side bar-graph depicts the frequencies of participants who were prescribed new medication for diabetes during a follow-up period of 36 months.

The majority of patients in the Indonesia DISCOVER study were initiated on metformin alone (36.7%), sulfonylurea alone (19.9%), or combination of these medications (10.9%). This was based on the HbA1c result or clinician's decision based on PERKINI guidelines.[14] As second-line therapy, metformin and sulfonylurea (26.7%) and metformin, sulfonylurea and DPP-4i (11.3%) were the most frequently prescribed combinations. The switch to second-line therapy was also consistent with some clinical guidelines.[3,5,9] After 3 years of follow-up, it was observed that metformin, sulfonylureas and basal insulin were used as diabetes treatments. Innovative oral anti-diabetic medications such as SGLT-2i and DPP-4i were used in only 1.2% and 1.1% of patients, respectively. A local study showed that 61.9% of patients received only oral anti-diabetic drugs (OAD), followed by insulin and OAD (19.4%), insulin monotherapy (17.3%), no pharmacologic treatment (1.1%) and herbal treatment (0.3%).[12] T2DM medications such as inhibitors of sodium-glucose cotransporter-2 (SGLT-2i) significantly reduce the risk of cardiovascular complications in high-risk patients, implying that treatment patterns may be beneficial to prevent further complications.[7,15-19] These newer and safer agents appear to be underutilized, maybe because they were more costly and unavailable in some provinces, despite their high efficacy and additional benefits for blood pressure, lipid and weight reduction, and cardio-renal outcomes.[16,17,19]

In Indonesia, 28% of patients with T2DM develop microvascular complications such as nephropathy (7.7%), neuropathy (17.6%) and retinopathy (2.7%). Whereas, 16.8% of patients develop macrovascular complications e.g., CAD (5.4%), heart failure (5%) and cerebrovascular disease (5.4%).[1] It is estimated that the relative risk of patients with T2DM for microvascular complications is at least 10–20 times greater compared to people without T2DM. Moreover, the risk for macrovascular disorders is about 2–4 times greater and accounts for approximately 65 percent of deaths.[5,20] Our study revealed that microvascular and macrovascular complications occurred at rates of 32% and 52%, respectively. Due to a lot of missing data on microvascular complications, these may be underestimated but enough to demonstrate the critical importance of early disease diagnosis, as recommended by guidelines.

The rate of complications in T2DM patients is concerning and warrants further investigation in Indonesia. T2DM and its complications have reached epidemic proportions, especially in developing countries.[1,21] The significant increase in the prevalence of diagnosed and undiagnosed T2DM, combined with advancements in diabetes treatment, has resulted in increased financial burden of diabetic complications; for example, 53% of the disease's lifetime medical costs have been attributed to treating complications. The critical importance of aggressive efforts to raise awareness, compliance to treatment, early diagnosis, and optimal monitoring to achieve treatment

goals are necessary to slow the progression of cardio-renal complications associated with T2DM.[22]

This study had major limitations. The recruited sample size was smaller than the calculated sample size (221 vs. 285 patients) due to a number of reasons. The study was multi-center and involved a number of physicians, hence, it was difficult to manage and follow-up all patients at 13 sites for a period of 36 months. The mortality rate was approximately 6% and lost to follow-up rate was 9%. Practical constraints such as lack of infrastructure, high proportions of missing data at centers, or unwillingness of centers to participate in observational research might have caused potential selection bias. Furthermore, the different sites had different standards of health facilities for the diagnosis and treatment of patients. Due to the differences in the modalities, the opinions and clinical judgement of the physician plays a vital role in the determination and classification of diabetes complications. Patients were randomly recruited during the index period of 2015-2016 only, such that their follow up ended in 2019. Thus, considering all these factors, the current scenario of T2DM in Indonesia may be slightly different than the findings reported in the present study but likely to reflect the routine clinical care in the country.

CONCLUSION

The DISCOVER study is a large global initiative that provides critical information about the real-world management of patients with T2DM. This is the first study in Indonesia that gives a picture of T2DM management over 3 years follow-up. Majority of patients had suboptimal glycemic and metabolic control. The characteristics of T2DM patients in Indonesia were elevated BMI, hypertension and hyperlipidemia, which were the most common comorbidities, and it showed the wide use of metformin and sulfonylurea for treatment. After 3 years of follow-up, HbA1c levels, vascular complications and other risk factors remained high. Increased awareness, early detection and intervention, as well as optimal initiation of available glucose-lowering medications and treatment of other risk factors are important to improve T2DM management and outcome in Indonesia.

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

CRediT Author Statement
DWS, RR, MRS, RPS, WOT: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft preparation and Writing – review and editing.

Author Disclosure
Dr. Made Ratna Saraswati is the JAFES Associate Editor of Indonesia. Dr. Widya Oktaviana Tarigan is the Medical Scientific Liaison of AstraZeneca Indonesia. Drs. Soeatmadji, Rosandi and Sibarani declared no conflicts of interest.

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ORIGINAL ARTICLE

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Patient Characteristics, Disease Burden, Treatment Patterns and Outcomes in Patients with Acromegaly: Real-World Evidence from the Malaysian Acromegaly Registry

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Abstract

Objective. This study aims to report the demographic features of patients with acromegaly, the disease burden, and the corresponding treatment patterns and outcomes in Malaysia.

Methodology. This is a retrospective study that included patients from the Malaysian Acromegaly registry who were diagnosed with acromegaly from 1970 onwards. Data collected included patient demographics, clinical manifestations of acromegaly, biochemical results and imaging findings. Information regarding treatment modalities and their outcomes was also obtained.

Results. Registry data was collected from 2013 to 2016 and included 140 patients with acromegaly from 12 participating hospitals. Median disease duration was 5.5 years (range 1.0 – 41.0 years). Most patients had macroadenoma (67%), while 15% were diagnosed with microadenoma. Hypertension (49.3%), diabetes (37.1%) and hypopituitarism (27.9%) were the most common co-morbidities for patients with acromegaly. Majority of patients had surgical intervention as primary treatment (65.9%) while 20.7% were treated medically, mainly with dopamine agonists (18.5%). Most patients had inadequate disease control after first-line treatment regardless of treatment modality (79.4%).

Conclusion. This registry study provides epidemiological data on patients with acromegaly in Malaysia and serves as an initial step for further population-based studies.

Key words: acromegaly, Malaysian registry, healthcare resource utilization, treatment outcomes

INTRODUCTION

Acromegaly is a rare endocrine disease resulting from excessive growth hormone (GH) production and affects both men and women equally.[1] In Western countries, the reported prevalence is approximately 70 to 80 cases per million population with an incidence of 3-11 cases per million population per year,[2-5] although the cases reported from Asia are lower at a prevalence of 28 and incidence of 4 cases per million population/year.[6]

Acromegaly is mainly caused by the presence of GH-secreting pituitary tumours. Elevated GH and insulin-growth factor 1 (IGF-1) result in anatomical changes and metabolic dysfunction.[1] Dysregulation of both hormones results in acromegaly-associated symptoms such as changes in facial appearance, overgrowth of hands and feet, headache, endocrine dysfunction and cardiovascular diseases.[7-11] Due to its insidious nature, the diagnosis of acromegaly is often delayed, at least 4 to 10 years after disease onset.[12-14] Unfortunately, diagnostic delays often lead to poor patient quality of life as well as increased morbidity and mortality.[15,16]

Clinical features that are suggestive of acromegaly such as headaches, diabetes mellitus, hypertension and heart disease of unknown aetiology warrant biochemical screening with GH and IGF-1 to confirm a diagnosis of

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acromegaly. Magnetic resonance imaging of the pituitary is also requested.[17] Main treatment goals include tumour removal and normalisation of GH and IGF-1 levels, accompanied by the resolution of clinical symptoms and eventually, a reduction in long-term mortality.[17] The main therapeutic options for acromegaly include surgical resection of adenomas, medical treatment with dopamine agonists and somatostatin analogues, and radiotherapy.[8,11,17] Transsphenoidal surgery is often considered the first-line treatment for most patients, with medical therapy being reserved as second-line treatment for patients who refuse surgery to control hormone production and tumour growth.[8,17-19]

Patient presentation, demographics and treatment modalities vary greatly amongst countries and regions. There was no prior data on acromegaly in Malaysia, hence, the Malaysian Endocrine and Metabolic Society (MEMS) established the acromegaly disease registry in 2013. Data were collected from 12 participating hospitals, including patients diagnosed with acromegaly from 1970 onwards. There is currently also no published literature on acromegaly in Malaysia. This study aims to report the demographic features of patients with acromegaly, the burden of the disease, the different treatment modalities and the corresponding treatment outcomes in Malaysia using data from the acromegaly disease registry.

METHODOLOGY

Data were collected from 2013 to 2016 from the Malaysian Acromegaly Registry with permission obtained from MEMS. Any patient diagnosed with acromegaly from 1970 onwards was included in the registry, whether they were identified at routine clinical appointments or upon reviewing hospital records. A diagnosis of acromegaly was made based on radiographic evidence of a pituitary adenoma plus any one of the following biochemical criteria: 1) Documented elevated IGF-1 level; 2) Failure of GH suppression with oral glucose tolerance test (OGTT); 3) Elevated GH levels in the presence of clinical features of acromegaly. Patients were excluded if either biochemical evidence or pituitary imaging was negative or unavailable.

Data collected included demographic, clinical (symptoms, signs, and comorbidities), biochemical and hormonal profiles, imaging results, as well as information regarding treatment modalities and their therapeutic outcomes. Outcomes were categorized as either controlled disease defined by an age-adjusted normal IGF-1 level and a random GH concentration <1 μg/L, or persistent disease if either GH or IGF-1 remained above the normal range.

All data was collected, stored and used in strict accordance with current Malaysia legislation on data protection, ethics and written informed patient consent. The study was approved by Malaysia Medical Research and Ethics Committee (MREC no: NMRR-12-1324-13746).

Statistical methods

In general, categorical variables were presented as count and percentages, while continuous variables were presented as count and median with range (min-max), stratified by tumour size.

RESULTS

A total of 140 patients with acromegaly from 12 participating hospitals were included in the registry. The median disease

Table 1. Patient baseline characteristics

Characteristics	Overall	Size of pituitary tumour*	
		Macro-adenoma	Micro-adenoma
Total patients, N (%)	140 (100.0)	95 (67.9)	21 (15.0)
Median age, years, (range)	52.0 (20.0 – 80.0)	49.0 (20.0 – 80.0)	58.0 (31.0 – 75.0)
Age groups at diagnosis, n (%)			
<18 years	0 (0.0)	0 (0.0)	0 (0.0)
18 - <30 years	11 (7.9)	9 (9.5)	0 (0.0)
30 - <50 years	51 (36.4)	39 (41.1)	6 (28.6)
≥50 years	77 (55.0)	46 (48.4)	15 (71.4)
Gender, n (%)			
Female	68 (48.6)	45 (47.4)	11 (52.4)
Male	70 (50.0)	48 (50.5)	10 (47.6)
n	138	93	21
Median Disease duration, years (range)	5.5 (1.0 – 41.0)	4.0 (1.0 – 41.0)	8.0 (1.0 – 19.0)
Categorized disease duration, n (%)			
<5 years	62 (44.3)	51 (53.7)	7 (33.3)
5 – 10 years	36 (25.7)	23 (24.2)	8 (38.1)
>10 years	40 (28.6)	19 (20.0)	6 (28.6)
n	94	77	13
Median IGF-1 at diagnosis, μg/L (range)	627.0 (4.5 – 1115.0)	601.0 (4.5 – 1115.0)	724.0 (400.0 – 892.0)
n	66	54	9
Median GH at diagnosis, measured post-OGTT, μg/L (range)	13.9 (0.2 – 730.0)	17.2 (0.2 – 730.0)	4.0 (1.0 – 36.0)
n	64	48	12
Median GH at diagnosis, measured post fasting, μg/L (range)	17.0 (0.0 – 104.0)	18.5 (0.2 – 104.0)	9.1 (0.2 – 32.9)
Clinical manifestations of acromegaly, n (%)			
Hypertension	69 (49.3)	40 (42.1)	15 (71.4)
Diabetes	52 (37.1)	33 (34.7)	10 (47.6)
Hypopituitarism	39 (27.9)	27 (28.4)	2 (9.5)
Dyslipidemia	19 (13.6)	13 (13.7)	4 (19.0)
Arthritis	17 (12.1)	13 (13.7)	3 (14.3)
Sleep apnea	10 (7.1)	6 (6.3)	3 (14.3)
Toxic MNG + Hyperthyroidism	10 (7.1)	9 (9.5)	1 (4.8)
Visual field detect	5 (3.6)	4 (4.2)	0 (0.0)
Thyroid Carcinoma	4 (2.9)	2 (2.1)	2 (9.5)
Co-secreting prolactin + hPL	5 (3.6)	5 (5.3)	0 (0.0)
Cardiac (LVH + MR)	3 (2.1)	3 (3.2)	0 (0.0)
Carpal Tunnel Syndrome	2 (1.4)	2 (2.1)	0 (0.0)
Renal Calculi	2 (1.4)	2 (2.1)	0 (0.0)
Osteoporosis	2 (1.4)	0 (0.0)	1 (4.8)
Stroke	1 (0.7)	0 (0.0)	1 (4.8)
Apoplexy	1 (0.7)	1 (1.1)	0 (0.0)
Migraine	1 (0.7)	1 (1.1)	0 (0.0)
Others	11 (7.9)	5 (5.3)	2 (9.5)

*Tumour size was not documented for 24 patients
Abbreviation: GH, growth hormone; hPL, human placental lactogen; IGF-I, insulin growth factor-I; LVH, left ventricular hypertrophy; MNG, multinodular goiter; MR, mitral regurgitation

Table 2. Primary treatment modalities

Characteristics	Overall	Size of pituitary tumour*	
		Macro-adenoma	Micro-adenoma
Total patients, N	140	95	21
Received primary healthcare treatment, M (%)	135 (96.4)	91 (95.8)	21 (100.0)
Type of treatment regimens, n (%)			
Single treatment modality	121 (89.6)	81 (89.0)	20 (95.2)
Medical	28 (20.7)	18 (19.8)	9 (42.9)
Surgery	89 (65.9)	61 (67.0)	11 (52.4)
Transfrontal	3 (2.2)	2 (2.2)	0 (0.0)
Transsphenoidal	84 (62.2)	58 (63.7)	11 (52.4)
Others or undefined	2 (1.5)	1 (1.1)	0 (0.0)
Radiotherapy	4 (3.0)	2 (2.2)	0 (0.0)
Conventional	2 (1.5)	2 (2.2)	0 (0.0)
Stereotactic	2 (1.5)	0 (0.0)	0 (0.0)
Others or undefined	0 (0.0)	0 (0.0)	0 (0.0)
Dual treatment modalities	12 (8.9)	10 (11.0)	0 (0.0)
Medical and surgery	8 (5.9)	8 (8.8)	0 (0.0)
Surgery and radiotherapy	4 (3.0)	2 (2.2)	0 (0.0)
Triple treatment modalities	2 (1.5)	0 (0.0)	1 (4.8)
Type of medical regimens, n (%)			
Single medication	34 (25.2)	24 (26.4)	8 (38.1)
Dopamine agonists	25 (18.5)	17 (18.7)	7 (33.3)
Somatostatin analogues	7 (5.2)	5 (5.5)	1 (4.8)
Others or undefined	2 (1.5)	2 (2.2)	0 (0.0)
Double medications	4 (3.0)	2 (2.2)	2 (9.5)
Dopamine agonists and Somatostatin analogues	4 (3.0)	2 (2.2)	2 (9.5)

*Tumour size was not documented for 24 patients
NB: Percentage calculated using denominator "received primary healthcare treatment, M"

Table 3. Current treatment modalities

Characteristics	Overall	Size of pituitary tumour	
		Macro-adenoma	Micro-adenoma
Total patients, N	140	95	21
Received current healthcare treatment, M (%)	71 (50.7)	49 (51.6)	14 (66.7)
Type of treatment regimens, n (%)			
Single treatment modality	71 (100.0)	49 (100.0)	14 (100.0)
Medical	67 (94.4)	46 (93.9)	14 (100.0)
Surgery	3 (4.2)	2 (4.1)	0 (0.0)
Transsphenoidal	1 (1.4)	1 (2.0)	0 (0.0)
Others or undefined	2 (2.8)	1 (2.0)	0 (0.0)
Radiotherapy	1 (1.4)	1 (2.0)	0 (0.0)
Others or undefined	1 (1.4)	1 (2.0)	0 (0.0)
Type of medical regimens, n (%)			
Single medication	62 (87.3)	43 (87.8)	13 (92.9)
Dopamine agonists	27 (38.0)	16 (32.7)	8 (57.1)
Somatostatin analogues	35 (49.3)	27 (55.1)	5 (35.7)
Double medications	5 (7.0)	3 (6.1)	1 (7.1)
Dopamine agonists and Somatostatin analogues	3 (4.2)	1 (2.0)	1 (7.1)
Others or undefined	2 (2.8)	2 (4.1)	0 (0.0)

NB: Percentage calculated using denominator "received primary healthcare treatment, M"

Table 4. First line treatment outcomes

	Treatment outcome*	
	Controlled disease (N=28)	Persistent disease (n=81)
First line treatment, n (%)		
Medical only	3 (10.7)	13 (16.0)
Surgical only	19 (67.9)	57 (70.4)
Radiotherapy only	1 (3.6)	3 (3.7)
Medical + surgical	4 (14.4)	3 (3.7)
Surgical + radiotherapy	1 (3.6)	3 (3.7)
Medical + surgical + radiotherapy	0	2 (2.5)

*There was no information on treatment outcome in 29 patients. 1 patient had deficiency in hormone levels post-removal of pituitary tumour.

duration was 5.5 years (range 1.0 – 41.0 years). Median age of patients was 52 years, with equal distribution across gender (female 68 [48.6%]; male 70 [50.0%]). Over 67% of patients were diagnosed with macroadenoma, while a minority of patients had microadenoma (n=21, 15%). Tumour size was not documented in 24 patients. IGF-1 levels were available for 94 patients at diagnosis, but only 66 patients had an OGTT with GH measurement at diagnosis.

Hypertension (49.3%), diabetes (37.1%) and hypopituitarism (27.9%) were the most common co-morbidities for patients with acromegaly. Patients with microadenoma were more frequently found to have hypertension and diabetes compared to those with macroadenoma (71.4% vs 42.1%; 47.6% vs 34.7%). Some patients with macroadenomas had co-secretion of prolactin and human placental lactogen (5%, 5.3% respectively). Unexpectedly, there were 2 cases of hypopituitarism (9.5%), which is not usually associated with microadenomas. The full list of clinical manifestations of acromegaly is presented in Table 1.

Regardless of adenoma size, majority of patients received monotherapy with most patients undergoing surgery alone (65.9%) while 20.7% had medical treatment alone as first-line intervention (Table 2). A minority of patients required dual therapy with either a combination of medical and surgical intervention (5.9%) or a combination of surgery and radiotherapy (3.0%). In terms of medical intervention, dopamine agonists were more frequently used (18.5%) in contrast to somatostatin analogues (5.2%).

Currently, the majority of the patients are receiving monotherapy with medical treatment being the dominant choice (94.4%). There are no patients on dual or triple therapy. Somatostatin analogues are used slightly more than dopamine agonists (49.3% vs 38.0%) (Table 3).

A total of 109 patients reported treatment outcomes after first-line treatment (Table 4). Regardless of the treatment modality, most patients had persistent disease.

DISCUSSION

The acromegaly registry was established to obtain epidemiological data on patients with acromegaly in Malaysia. It was funded and supported by MEMS. From this current study, data collected between 2013 and 2016 showed that there were only 140 patients listed in the registry were diagnosed with acromegaly from 1970 onwards. The Malaysian Consensus Statement for the Diagnosis and Management of Acromegaly highlights the condition as an underrecognised and underdiagnosed condition in the country.[17] Clinicians face major challenges in management due to the delayed diagnosis.[20-22] Median duration of disease to diagnosis was 5.5 years, although nearly a third were only diagnosed after 10 years, with a majority of patients having macroadenomas (68%). As a result of this finding, a clinical pathway for screening and subsequent referral to the nearest, most accessible endocrinologist and endocrine center was formulated.

There is also a minority of patients who did not have any information regarding tumour status; it cannot be derived from the registry if the tumour status was undocumented or if MRI/CT imaging was not done. Hypertension, diabetes, hypothyroidism and dyslipidemia were the most commonly reported symptoms of acromegaly among patients in Malaysia, which concurs with published reports and other Asian acromegaly registry studies.[10,20-22] Interestingly, two patients with microadenoma had hypopituitarism, which is usually associated with macroadenomas. There have been previous reports on cases of microadenomas with resultant hypopituitarism.[23,24] Although, patients with acromegaly frequently have multinodular non-toxic goiters, some present with less common thyroid presentations such as toxic multinodular goiter (10 patients) and thyroid carcinoma (4 patients). A recent meta-analysis concerning different cancer types in acromegaly reported a significant increase in the prevalence of thyroid cancers.[25]

In the past, endocrinology practice in Malaysia was confined to a limited number of certain public hospitals which hampered the screening of these patients, ultimately resulting in delayed diagnosis and management. With the increasing number of endocrinologists, more cases are expected to be detected and referred for complete assessment and long-term care.

From this study, only around 67% of patients had IGF-1 results. IGF-1 assay only became available in Malaysia in 1993 and was previously only available at the Institute of Medical Research with a long turnaround time. Currently, selected public hospitals also offer IGF-1 assays which should increase the availability of this test.

Surgery is the treatment of choice for most patients (76.3%), especially for those with macroadenomas. As per guidelines[17], transsphenoidal surgical removal of adenomas is the preferred technique for most patients. Clinical practice in Malaysia concurred with practice in other Asian countries such as Japan, Korea, China and Taiwan.[20-22,26]

In terms of medical treatment, guidelines recommend the use of somatostatin analogues first and consider dopamine agonists in milder cases.[17-19] However, dopamine agonists are given more often used than somatostatin analogues in practice due to its ease of administration, wide accessibility and affordability. In Malaysia, the use of somatostatin analogues is restricted and tightly regulated. It is mainly reserved as second-line treatment when surgery or dopamine agonists have failed to control the disease. Although the GH receptor antagonist, pegvisomant is currently the most effective treatment for acromegaly,[27] it is currently unavailable in Malaysia.

The use of radiotherapy is low in Malaysian patients because it is usually reserved for residual or recurrent tumours with high surgical risk or if the patient refuses surgery.[17] Data from the registry shows that additional radiotherapy was given only if with persistent disease after primary treatment. However, it has posed a greater risk of hypopituitarism and an uncertain long-term complication rate.[21] Although radiotherapy has taken a back seat with the development of effective and safer medical therapies such as somatostatin analogues or pegvisomant, radiotherapy still plays a role in salvage therapy.[17-19,28-30]

Regardless of the treatment modality, a high proportion of patients continue to have persistent disease. A recent study looking at acromegaly in Central and Eastern Europe, Israel and Kazakhstan also showed that there could be improvements in disease control.[31] Chronic exposure to elevated levels of GH and IGF-1 prior to diagnosis and optimal treatment of disease are suggested to play an important role in disease persistence.[32] However, with the limitation in the registry data, we are unable to suggest any correlation of patient characteristics to disease persistence.

One limitation of this study was that only 12 hospitals were invited to participate in this registry and therefore, may not accurately capture the full picture of all patients with acromegaly in Malaysia. However, these are the main endocrine centers in the public sector. A few patients were seen privately but most patients were referred to these public endocrine centers for long-term follow-up. Data may be incomplete since only those submitted by clinicians would be included in the registry.

Data presented were collected before the Malaysia Consensus Statement for the Diagnosis and Management of Acromegaly was launched in 2019.[17] Since the consensus was launched, there have been huge initiatives to standardise diagnosis and management of acromegaly in Malaysia, including efforts to increase awareness of the disease among primary care physicians. These efforts have also reached out to other healthcare professionals, patients and caregivers. A patient support group was established for patients with acromegaly. A future study is currently underway to assess the impact of the consensus and these initiatives.

CONCLUSION

This registry data provided the first epidemiological snapshot of patients with acromegaly in Malaysia serving as an initial step for further population-based studies. This study serves as baseline of the clinical practice of diagnostics, treatment and management of patients with acromegaly. With the establishment of the consensus statement, we hope that future initiatives would help improve patient care.

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

CRediT Author Statement
MBLB: Conceptualization; Methodology, Investigation, Resources, Data curation, Writing – review and editing, Supervision, Project administration; **AMK:** Conceptualization, Resources, Writing – review and editing, Visualization, Project administration, Funding acquisition; **FHST:** Investigation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision; **NAA:** Investigation, Resources, Writing – review and editing; **NMA:** Investigation, Writing – original draft preparation, Writing – review and editing; **NAK:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Supervision; **SRV:** Writing – original draft preparation, Writing – review and editing; Visualization; **BS:** Conceptualization, Project administration, Funding acquisition; **ZH:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition.

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ORIGINAL ARTICLE

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Association of Vitamin D levels on the Clinical Outcomes of Patients Hospitalized for COVID-19 in a Tertiary Hospital

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Abstract

Objectives. This study aimed to compare the severity of COVID-19, inflammatory parameters and clinical outcomes among patients with normal and subnormal levels of Vitamin D.

Methodology. This is a retrospective cohort study of 135 patients admitted in a tertiary hospital for COVID-19. Patients were grouped according to their Vitamin D level. Primary outcome measure was the composite of all-cause mortality and morbidity. Other outcome measures determined were the comparison among the groups on the severity of COVID-19 infection, changes in inflammatory parameters, length of hospital stay and duration of respiratory support.

Results. There was a significant trend of higher ICU admission (p=0.024), mortality (p=0.006) and poor clinical outcome (p=0.009) among the Vitamin D deficient group. No significant difference was found for most of the inflammatory parameters, duration of hospital stay and respiratory support. Overall, patients with deficient, but not insufficient Vitamin D level had 6 times higher odds of composite poor outcome than those with normal Vitamin D (crude OR=5.18, p=0.003; adjusted OR=6.3, p=0.043).

Conclusion. The inverse relationship between Vitamin D level and poor composite outcome observed in our study suggests that low Vitamin D may be a risk factor for poor prognosis among patients admitted for COVID-19.

Key words: Vitamin D, Vitamin D deficiency, COVID-19

INTRODUCTION

Vitamin D deficiency is a public health problem affecting over a billion people among different age-groups worldwide.[1] Based on a 2013 National Nutrition Survey, Filipino adults have a high prevalence of Vitamin D insufficiency.[2] Vitamin D has a wide range of functions, not only in bone health but also in enhancement of the immune system. Vitamin D receptor (VDR) is present in most tissues including the immune system and calcitriol [$1,25(OH)_2D_3$], the active form of Vitamin D, can also be synthesized by antigen-presenting cells and lymphocytes where it inhibits the expression of inflammatory cytokines.[3,4] Several studies demonstrated the role of Vitamin D in reducing the risk of acute viral respiratory tract infections, including direct inhibition of viral replication or through anti-inflammatory or immunomodulatory functions.[4,5] Individuals with lower Vitamin D levels were more likely to have upper respiratory tract infection than those with adequate levels, even after adjusting for variables including season, age, gender, body mass index (BMI) and race.[4] During the COVID-19 pandemic, people with Vitamin D deficiency could be at higher risk of acquiring a more severe type of disease and Vitamin D supplementation has been hypothesized to be an effective means of preventing the worsening of disease course caused by SARS-CoV-2.[5-7]

It has been demonstrated that the human DPP-4/CD26 receptor, a virulence factor in COVID-19 infection, can be reduced by adequate levels of vitamin D.[6] The bioavailability and expression of angiotensin-converting enzyme 2 (ACE2), which is responsible for inactivating the virus, are both enhanced by Vitamin D.[8] Acute lung injury and other organ failures in COVID-19 are caused by activation of the renin-angiotensin-aldosterone system (RAS), and Vitamin D can act as a negative RAS regulator and inducer of ACE2 to mitigate the effects of the virus.[8] Upon correction of the Vitamin D deficiency, the immunological changes could begin within a short period, as immune responses are dynamic processes that protect against viral infections.[4] However, data is still conflicting on whether Vitamin D supplementation can be used as prophylaxis or adjunctive therapy in COVID-19.

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To date, there is no local data regarding the association of Vitamin D levels with the severity of COVID-19 infection. This study aimed to compare the severity of COVID-19, inflammatory parameters and clinical outcomes among patients with normal and subnormal levels of Vitamin D, and to determine the association of Vitamin D level with the all-cause mortality and morbidity among patients admitted due to COVID-19.

METHODOLOGY

This is a retrospective study that utilized chart review of COVID-19 patients admitted at Chinese General Hospital and Medical Center from January 2021 to August 2021, which were managed by or referred to Endocrinology service. Included patients were at least 18 years old, confirmed positive for COVID-19 by RT-PCR and with Vitamin D assay [25(OH)D] done upon admission or within 2 weeks from date of admission. Exclusion criteria include pregnant patients, those with granulomatous disorders, on medications that may affect Vitamin D metabolism (chronic glucocorticoid use, antiseizure medications, antiretroviral medication such as efavirenz and zidovudine), malabsorption syndromes, hyperparathyroidism and chronic kidney disease (CKD) on dialysis.

Patients were categorized according to 25(OH)D levels into normal (≥30 ng/ml; SI: >75 nmol/L), insufficient (21–29 ng/ml; SI:50-75 nmol/L) or deficient (<20 ng/ml; SI: <50 nmol/L) group as defined by the Endocrine Society Guidelines.[9] Primary outcome investigated was the composite of all-cause mortality and morbidity which included respiratory decompensation (the need for invasive or increase in requirement of noninvasive ventilation), cardiac decompensation (the need for vasopressors or episodes of pulseless rhythms) or acute kidney failure with the need for renal replacement therapy and ICU admission. Other outcomes investigated include changes in inflammatory parameters [ferritin, procalcitonin PCT), C-reactive protein (CRP), lactate dehydrogenase (LDH) and D-dimer], length of stay of hospitalization (LOS) and duration of respiratory support.

Laboratory tests for 25(OH)D, ferritin and PCT were measured with Abbott Architect machine, while LDH and CRP with Beckman Coulter using the principle of chemiluminescence immunoassay; D-dimer was measured with STA Compact Max using the principle of photometry. Comparison was made between the groups in terms of severity of COVID-19 infection and changes in inflammatory parameters (ferritin, PCT, CRP, LDH, D-dimer). Severity of COVID-19 was defined using the World Health Organization (WHO) Classification used in the clinical management of COVID-19 Interim Guidance 2020[10] (Supplemental Table B).

Sample size

We estimated the sample size of at least 83 patients for the study population. This was based on 80% statistical power, 95% confidence level and 94.3% prevalence of Vitamin D deficiency among COVID-19 patients based on a study by Demir et al.[11] The current study included all admitted COVID-19 patients with Vitamin D assay that were referred to or managed by the Section of endocrinology for the specified time.

Data analysis

All data were encoded in MS Excel and Stata MP version 16 software was used for data processing and analysis. Continuous variables were presented as mean (standard deviation/SD) or median (interquartile range/IQR) depending on the data distribution. Categorical variables were presented as frequencies and percentages. One-way ANOVA or Kruskal Wallis test was performed to compare continuous variables. Significant Kruskal Wallis test was further analyzed using Dunn's test. Chi-square test or Fisher's exact test was used to analyze categorical variables.

In order to determine the association between Vitamin D level and composite poor outcome, logistic regression analysis with Firth's bias correction was done. Screening for potential confounders was performed using simple logistic regression analysis and a cutoff of $p<0.20$[12] (Supplemental Table A). Model building was performed using multiple logistic regression analysis and significant confounders were retained in the model using the change-in-estimate criterion of 10%. Imputation for missing data was not performed. $P \leq 0.05$ were considered statistically significant.

Ethical Considerations

This study adhered to the ethical considerations and ethical principles set out in relevant guidelines, including the Declaration of Helsinki, WHO guidelines, International Conference on Harmonization-Good Clinical Practice, Data Privacy Act of 2012 and National Ethics Guidelines for Health Research 2017. The study commenced upon the approval of the Chinese General Hospital and Medical Center Research Ethics Review Board (CGHMC RERB2021-F-52). The results and patient information were kept strictly confidential by the primary investigator. All data were encoded using a password-protected spreadsheet.

RESULTS

A total of 137 charts were retrieved. Two patients were excluded due to the use of antiseizure medications and comorbidity of CKD on dialysis. Among 135 patients analyzed, the median Vitamin D level was 25ng/ml (SI:50nmol/L) [IQR: 19.8-31; Range: 6.7-73]. Only 38 (28.2%) patients had normal Vitamin D level. Of those with abnormal result, 63 (46.7%) had insufficiency, and 34 (25.2%) had deficiency.

Table 1 shows the baseline characteristics of patients including the comorbidities. Most patients were 60-75 years old, male and had moderate COVID-19 infection.

Majority had normal BMI for each group (*Normal=84.2%, Insufficient=76.2%, Deficient=44.1%*). However, there was a higher percentage of overweight (29.4%) and obesity (23.5%) among the Vitamin D deficient group. BMI (p=0.0005), type 2 diabetes mellitus (T2DM) (p=0.013), heart failure (p=0.036) and COVID severity (p=0.045) (Figure 1) significantly differ by Vitamin D level. In terms of severity of infection, more patients had severe and critical illness with hypovitaminosis D compared to normal Vitamin D group (p=0.045) (Figure 1).

For the baseline inflammatory markers, only median CRP was significantly different by Vitamin D level. Further analysis using Dunn's test revealed that median CRP of patients with deficient Vitamin D was significantly higher compared to normal (p=0.0070) and insufficient (p=0.0111)

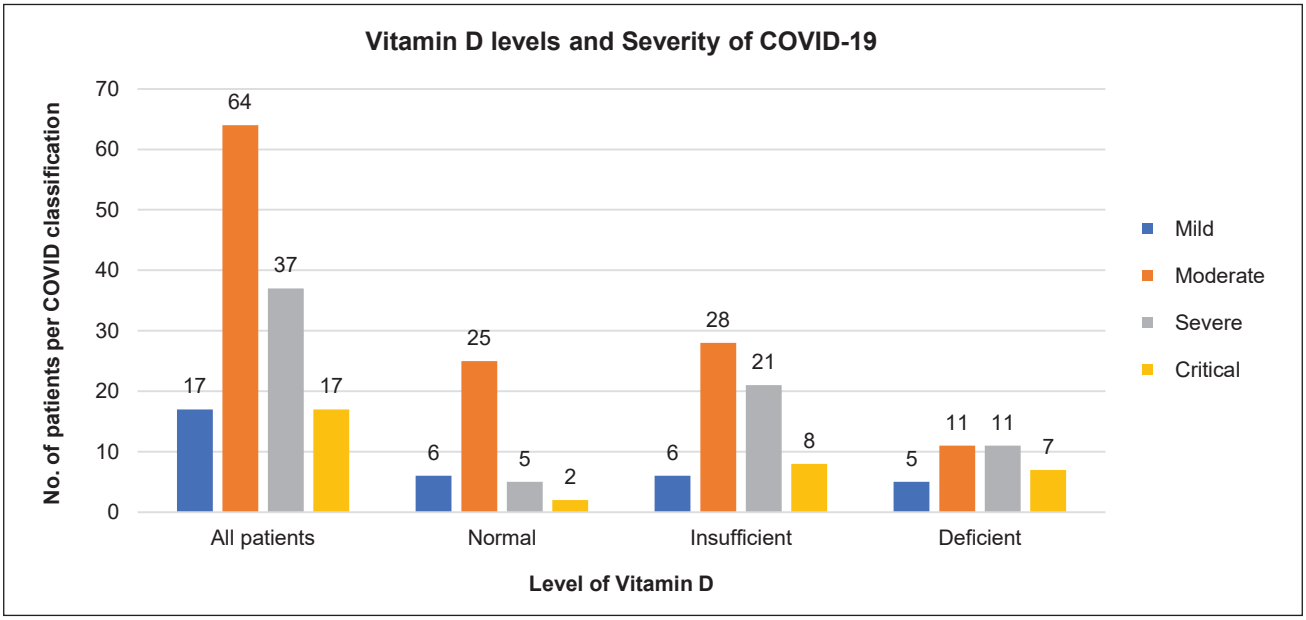

Figure 1. Severity of COVID-19 infection in relation to levels of Vitamin D. In terms of severity of the disease, more patients have severe and critical illness with hypovitaminosis D compared to normal Vitamin D group (p=0.045).

Table 1. Baseline demographic and clinical characteristics by Vitamin D level (n=135)

	All patients (n=135) n (%)	Normal Vitamin D >30 ng/ml (n=38) n (%)	Insufficient Vitamin D 21-29 ng/ml (n=63) n (%)	Deficient Vitamin D <20 ng/ml (n=34) n (%)	*p*
Age (in years), mean	64.8 ± 14.0	67.8 ± 12.0	62.8 ± 14.7	65.3 ± 14.4	0.2102[a]
18-39 years old	6 (4.4)	0 (0.0)	4 (6.4)	2 (5.9)	0.684[b]
40-59 years old	39 (28.9)	10 (26.3)	21 (33.3)	8 (23.5)	
60-75 years old	62 (45.9)	19 (50.0)	26 (41.3)	17 (50.0)	
Above 75 years old	28 (20.7)	9 (23.7)	12 (19.0)	7 (20.6)	
Sex					
Female	50 (37.0)	16 (42.1)	25 (39.7)	9 (26.5)	0.327[c]
Male	85 (63.0)	22 (57.9)	38 (60.3)	25 (73.5)	
Asia-Pacific BMI (in kg/m^2), median	23 [IQR: 21-26]	22 [IQR: 20-23]	22 [IQR: 21-25]	26.4 [IQR: 22-30]	0.0005*[d]
Underweight (18.5)	2 (1.5)	1 (2.6)	0	1 (2.9)	0.001*[b]
Normal (18.5-22.9)	95 (70.4)	32 (84.2)	48 (76.2)	15 (44.1)	
Overweight (23-24.9)	25 (18.5)	5 (13.2)	10 (15.9)	10 (29.4)	
Obese (≥25)	13 (9.6)	0	5 (7.9)	8 (23.5)	
Co-morbid condition, % with					
Hypertension	82 (60.7)	26 (68.4)	38 (60.3)	18 (52.9)	0.404[c]
Diabetes Mellitus	125 (92.6)	38 (100.0)	54 (85.7)	33 (97.1)	0.013*[b]
Cerebrovascular Disease	8 (5.9)	2 (5.3)	5 (7.9)	1 (2.9)	0.732[b]
Ischemic Heart Disease	10 (7.4)	5 (13.2)	4 (6.4)	1 (2.9)	0.243[b]
Heart Failure	3 (2)	3 (7.9)	0 (0.0)	0 (0.0)	0.036*[b]
Chronic Kidney Disease (not on Dialysis)	14 (10.4)	3 (7.9)	6 (9.5)	5 (14.7)	0.628[b]
Chronic Lung Disease (Asthma, COPD)	2 (1.5)	1 (2.6)	0 (0.0)	1 (2.9)	0.283[b]
Thyroid Diseases	13 (9.6)	3 (7.9)	7 (11.1)	3 (8.9)	0.931[b]
Malignancy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Tuberculosis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Chronic Liver Disease	3 (2)	1 (2.6)	2 (3.2)	0 (0.0)	0.797[b]
Others	3 (2)	1 (2.6)	1 (1.6)	1 (2.9)	1.000[b]

[a]One Way ANOVA was used; [b]Fisher's exact test was used; [c]Chi-square test was used; [d]Kruskal-Wallis test was used. Significant results were further analyzed using Dunn's test

group. No significant difference in median CRP was found between normal and insufficient Vitamin D (p=0.3317). Baseline and final results for the inflammatory markers were obtained and the median of these changes were compared among the 3 groups as shown in Table 2. Only changes in D-dimer and CRP significantly differ by Vitamin D level. Further analysis using Dunn's test showed that the median change (i.e., decrease) in D-dimer of patients with normal Vitamin D was significantly higher compared to insufficient Vitamin D level (p=0.0032), but not with deficient group (p=0.1542). Only 65.4% of patients with deficient Vitamin D had a decrease in CRP compared to 89.7% of normal Vitamin D level, and 86% of insufficient Vitamin D.

Overall incidence of poor clinical outcome was 34.8% (95% CI: 27.2-43.3% p=0.009). Clinical outcome was significantly different by Vitamin D level, such that more than half of patients with deficient Vitamin D had poor outcome compared to only 18.4% of those with normal Vitamin D (Table 3). Of the specific poor outcomes of interest, respiratory decompensation and initiation of renal replacement were not significantly different across the 3 groups. Compared to those with normal Vitamin D levels, a higher proportion of insufficient and deficient Vitamin D levels required vasopressor or inotropic support. None of the patients in the normal and insufficient Vitamin D groups had episodes of pulseless electrical activity or

Table 2. Change in inflammatory parameters by Vitamin D level

	All patients (n=135) Median [IQR]	Normal Vitamin D >30 ng/ml (n=38) Median [IQR]	Insufficient Vitamin D 21-29 ng/ml (n=63) Median [IQR]	Deficient Vitamin D <20 ng/ml (n=34) Median [IQR]	*p*
Ferritin (ng/mL) (n=131)	287.0 [IQR: 25.0-851.0]	341.0 [IQR: 86.0-823.0]	292.5 [IQR: 15.0-1164.0]	230 [IQR: -238-851]	0.8545[a]
Increased	5 (13.5)	5 (13.5)	12 (20.0)	9 (26.5)	0.392[b]
Decreased	32 (86.5)	32 (86.5)	48 (80.0)	25 (73.5)	
LDH (U/L) (n=124)	209.5 [IQR: 48.5-392.5]	298.5 [IQR: 120.0-532.0]	149.0 [IQR: 31.0-277.0]	198.0 [IQR: -7.0-285.0]	0.0535[a]
Increased	27 (21.8)	5 (13.9)	13 (23.6)	9 (27.3)	0.366[b]
Decreased	24 (78.2)	31 (86.1)	42 (76.4)	24 (72.7)	
D-dimer (ug/ml) (n=122)	0.1 [IQR: -0.3-2.0]	0.3 [IQR: 0.1-0.9]	0 [IQR: -0.6-0.4]	0.2 [IQR: -0.9-0.9]	0.0223*[a]
Increased	41 (33.6)	5 (13.5)	26 (46.4)	10 (34.5)	0.004*[c]
Decreased	80 (65.6)	32 (86.5)	29 (51.8)	19 (65.5)	
No change	1 (0.8)	0	1 (1.8)	0	
Procalcitonin (ng/ml) (n=106)	0.1 [IQR: 0.0-0.3]	0.1 [IQR: 0.0-0.4]	0.1 [IQR: 0.0-0.2]	0.1 [IQR: -0.1-0.3]	0.7489[a]
Increased	21 (19.8)	3 (10.7)	10 (20.0)	8 (28.6)	0.333[c]
Decreased	83 (78.3)	25 (89.3)	38 (76.0)	20 (71.4)	
No change	2 (1.9)	0	2 (4.0)	0	
C-reactive Protein (mg/L) (n=98)	3.5 [IQR: 1.2-5.5]	3.5 [IQR: 1.8-6.4]	3.7 [IQR: 1.4-5.5]	1.4 [IQR: 0-4.7]	0.1687[a]
Increased	14 (14.3)	3 (10.3)	6 (14.0)	5 (19.2)	0.025*[c]
Decreased	80 (81.6)	26 (89.7)	37 (86.0)	17 (65.4)	
No change	4 (4.1)	0	0	4 (15.4)	

[a]Kruskal-Wallis test was used. Significant results were further analyzed using Dunn's test; [b]Chi-square test was used; [c]Fisher's exact test was used

Table 3. Clinical outcomes by Vitamin D level (n=135)

	All patients (n=135) n (%)	Normal Vitamin D >30 ng/ml (n=38) n (%)	Insufficient Vitamin D 21-29 ng/ml (n=63) n (%)	Deficient Vitamin D <20 ng/ml (n=34) n (%)	*p*
Poor clinical outcome	47 (34.8)	7 (18.4)	22 (34.9)	18 (52.9)	0.009*[a]
Respiratory decompensation	38 (28.2)	7 (18.4)	18 (28.6)	13 (38.2)	0.174[a]
Requirement for vasopressor and/or inotropic support	28 (20.7)	2 (5.3)	13 (20.6)	13 (38.2)	0.003*[a]
Ventricular tachycardia or fibrillation/Pulseless Electrical Activity/ Resuscitated cardiac arrest	3 (2.2)	0 (0.0)	0 (0.0)	3 (8.8)	0.015*[b]
Initiation of renal replacement therapy	7 (5.2)	0	4 (6.4)	3 (8.8)	0.217[b]
Death from any cause	20 (14.8)	1 (2.6)	9 (14.3)	10 (29.4)	0.006*[a]
Length of hospital stay, all patients	12.0 [IQR: 9.0-17.0]	11.5 [IQR: 9.0-15.0]	12.0 [IQR: 9.0-17.0]	13.0 [IQR: 8.0-19.0]	0.7424[c]
Length of hospital stay, survivors (n=115)	11.0 [IQR:9.0-16.0]	11.0 [IQR: 9.0-15.0]	11.0 [IQR: 9.0-16.0]	12.0 [IQR: 7.0-18.5]	0.9488[c]
ICU admission					
No	112 (83.0)	36 (94.7)	52 (82.5)	24 (70.6)	0.024*[a]
Yes	23 (17.0)	2 (5.3)	11 (17.5)	10 (29.4)	
Length of ICU stay, median (IQR) (n=23)	14 [IQR: 13-22]	11 [IQR: 8-14]	17 [IQR: 14-30]	16 [IQR: 13-21]	0.3558[c]
Respiratory support (n=134)					
No	9 (6.7)	3 (7.9)	4 (6.4)	2 (6.1)	1.000[b]
Yes	125 (93.3)	35 (92.1)	59 (93.6)	31 (93.9)	
Length on respiratory support, median (IQR) (n=125)	10 [IQR: 7-14]	8 [IQR: 6-10]	10 [IQR: 7-14]	13 [IQR: 6-17]	0.0550[c]
Non-invasive					
Nasal cannula (n=64)	7 [IQR: 6-10]	7 [IQR: 6-9]	8 [IQR: 6.5-10]	6 [IQR: 6-8]	0.3159[c]
Face mask (n=7)	10 [IQR: 7-13]	13 [IQR: 10-16]	8 [IQR: 7-9.5]	13 [IQR: 13-13]	0.1503[c]
HFNC (n=36)	14 [IQR: 11-21]	10 [IQR: 7-15]	13.5 [IQR: 11-22]	14 [IQR: 14-25]	0.1570[c]
Invasive					
Mechanical Ventilation (n=18)	16.5 [IQR: 13-21]	20 [IQR: 20-20]	17 [IQR: 14-24]	15 [IQR: 13-21]	0.8371[c]

[a]Chi square test was used; [b]Fisher's exact test was used; [c]Kruskal-Wallis test was used

Table 4. Association between Vitamin D level and composite poor outcome (n=107)

	Crude OR (95% CI)	*p*	Adjusted OR (95% CI)[a]	*p*
Vitamin D level				
Normal (> 30 ng/ml)	Ref	Ref	Ref	Ref
Insufficient (21-29 ng/ml)	2.326 (0.858-6.307)	0.097	2.634 (0.566-12.261)	0.217
Deficient (<20 ng/ml)	5.174 (1.717-15.588)	0.003*	6.331 (1.059-37.836)	0.043*

Ref: Reference category; [a]Adjusted for the significant confounding effects of age, COVID-19 severity, hypertension, ischemic heart disease, chronic lung disease, baseline D-dimer, baseline LDH, and baseline procalcitonin

resuscitated cardiac arrest but this outcome was observed in 8.8% of Vitamin D deficient patients.

Overall mortality for the study population was 14.8%. There was a significant trend of higher mortality for the deficient group (p=0.006), while only 1 patient with normal Vitamin D died. There was no significant difference with the duration of hospitalization and the median LOS among the survivors was 11 days, (range of 5-45 days). Twenty-three patients (17%) in this study were admitted to the ICU. The proportion significantly differ by Vitamin D level such that only 5.3% of those with normal Vitamin D were admitted to ICU compared to 17.5% of insufficient and 29.4% of deficient levels. The type of respiratory support used and the length of respiratory support were not significantly different by Vitamin D level.

For the determination of the association between Vitamin D level and composite poor outcome, only 107 patients with complete data for all variables of interest were included. Vitamin D deficiency was significantly associated with composite poor outcome even after controlling for significant confounders (Table 4). Patients with deficient Vitamin D level had about 5 times higher odds of poor outcome than those with normal Vitamin D level (OR=5.177). After controlling for the effects of confounders, patients with deficient vitamin D had 6 times higher odds of poor outcome than patients with normal Vitamin D levels (adjusted OR = 6.3).

DISCUSSION

This is the first study in the Philippines to have investigated the association of Vitamin D levels with the clinical outcomes of COVID-19 infection. Majority of the subjects in our study had T2DM, which is the most common reason for COVID-19 referrals to the endocrinology service. Most of the baseline characteristics were comparable among the groups except for T2DM, obesity and heart failure; however these findings may not reflect data in the general population. Vitamin D deficiency has been shown to modify insulin synthesis and secretion, not only through regulation of plasma calcium, but also through a direct action on pancreatic beta-cell function, suggesting its pathogenicity for T2DM.[13] High prevalence of Vitamin D deficiency in subjects with above normal BMI is a well-documented finding. Causes include volumetric dilution into the greater volumes of fat, serum, liver or muscles, impaired hepatic 25-hydroxylation and possibly due to sequestration and altered metabolism in adipose tissues.[14] Hypovitaminosis D is also implicated in numerous cardiovascular diseases.[15] However, there was limited number of patients with documented ischemic heart disease or heart failure in our study.

In our results, there were more patients with deficient Vitamin D among the critical group and subnormal 25(OH) D levels for the severe group. This finding is similar to a prospective study by Campi et al., in which low 25(OH)D levels at hospital admission were associated with increased interleukin-6 (IL-6) levels and predicted both the disease severity and mortality during the course of hospitalization, independently of other comorbidities. They even suggested that 25(OH)D can be a considered as a useful prognostic marker for COVID- 19.[16] This study also supports the meta-analysis by Pereira et al., that severe type of cases had more individuals with deficient Vitamin D levels compared to mild cases, especially among the elderly.[17]

Vitamin D enhances cellular innate immunity partly through the induction of antimicrobial peptides, including human cathelicidins and defensins, thereby reducing the cytokine storm and severity of infection. Administering Vitamin D reduces the expression of pro-inflammatory cytokines and increases the expression of anti-inflammatory cytokines.[9] Human DPP-4/CD26, the target of the S1 domain of the COVID-19 spike glycoprotein, has been suggested as a virulence factor in COVID-19 infection. The expression of the DPP-4/CD26 is reduced significantly in vivo upon the correction of Vitamin D insufficiency and there is evidence that Vitamin D may reduce some of the negative immunological consequences.[8]

According to some studies, serum concentrations of 25(OH)D were inversely associated with pro-inflammatory cytokines, IL-6, CRP and the risk of pneumonia, acute respiratory distress syndrome (ARDS), diabetes and heart failure.[18,19] Biomarkers have been used for confirming and classifying disease severity in COVID-19 and according to some investigators, CRP was elevated in 60.7% of patients, PCT in 5.5%, and LDH in 41% of patients.[19] Patients with severe COVID-19 have a higher incidence of elevated CRP than those with a mild form of disease. Although CRP is a nonspecific marker, it becomes more specific to the bioactivity of IL-6 and formation of a cytokine storm in patients with severe COVID-19. Analysis of Vitamin D status and CRP levels among affected patients suggests that subjects with Vitamin D deficiency have more incidence of elevated CRP and cytokine storm than patients with normal Vitamin D status.[20]

In this study, only the baseline CRP and downward trend of CRP and D-dimer levels showed a significant difference between the 3 groups. D-dimer elevation signify a hyper-fibrinolysis state and increased inflammatory burden, worsening respiratory problems induced by COVID-19 infection. Significantly higher levels are found in those with critical illness and is used as a prognostic marker for in-hospital mortality or severity of COVID-19 infection.[21,22] Vitamin D metabolites have the ability to regulate different pro- and anti-thrombotic agent of coagulation cascade and some authors proposed that Vitamin D supplementation along with prophylactic anticoagulant can be potentially useful for COVID-19 associated coagulopathy.[23]

Studies on ferritin levels in COVID-19 patients have yielded equivocal results. Two retrospective studies have reported a minimal role of ferritin in predicting ICU admission and need for ventilation and failed to predict mortality.[19] Another study showed findings to the contrary, that ferritin levels could predict severe disease and mortality.[18] A small retrospective study revealed that ferritin was the last parameter to return to normal making it a marker of severity and less useful for monitoring the course of disease.[24] In our investigation, the insignificant baseline levels of ferritin, LDH and procalcitonin may reflect an earlier course of their disease, while the insignificant decrease in markers from the initial results could be due to coexisting infections or comorbidities and that other repeated levels of inflammatory parameters (between baseline and final values) were not taken into account during the course of their hospitalization.

Increased generation of pro-inflammatory cytokines in COVID-19 are responsible for the development of ARDS. The SARS-CoV-2 does not only affect the respiratory system but can also cause acute myocardial or kidney injury. In this study, among the specific clinical outcomes (Table 3), there was a higher trend of cardiac decompensation among the hypovitaminosis D groups. VDR is particularly prevalent in vascular smooth muscle and myocardium. Vitamin D, as an anti-inflammatory, can modulate nitric oxide (NO) production and inhibit endothelial protein expression for leukocyte adhesion; while as an antithrombotic, plays a role in the upregulation of thrombomodulin. Vitamin D also has an anti-remodeling effect, it decreases pro-inflammatory cytokines, reducing the fatality risk of obesity and heart failure among COVID-19 patients.[25]

Countries with below average Vitamin D levels have higher numbers of COVID-19 cases and mortality,[26] and hospital LOS was significantly shorter in the sufficient group than in the deficient group suggesting that treatment of Vitamin D deficiency can prevent COVID-19 severity or death.[17,20] In another prospective study, among hospitalized patients with moderate to severe COVID-19, those with severe 25(OH)D deficiency (<10 ng/mL) exhibited a trend for longer hospital LOS, although not statistically significant, compared with patients with higher 25(OH)D concentrations.[27] Other observational studies also lack significant association of hypovitaminosis D with ICU admission, type and length of respiratory support, need for mechanical ventilation and mortality.[28,29] Conflicting results may be due to a smaller sample size in some studies, presence of bacterial co-infections and differences in study design and cut-off for categorizing Vitamin D level. Our investigation did not reveal statistically significant difference among the type and length of respiratory support and LOS. These could be due to smaller sample size and that majority of the subjects had moderate COVID-19. Medications used for COVID-19 and different patterns of weaning from respiratory support by various infectious disease specialists and pulmonologists could have also affected the results.

Overall, congruent with other studies, our research showed that serum 25(OH)D levels were significantly associated with risk of ICU admission, COVID-19-related in-hospital mortality and with the composite poor outcome. Furthermore, it was found to be associated with CRP and D-dimer which are among the most useful predictive biomarkers for poor prognosis of COVID-19.[6,23,27,30] These findings suggest the role of Vitamin D in suppressing some of the inflammatory markers in cytokine storm that are responsible for the poor clinical outcome or mortality among COVID-19 patients. Whether Vitamin D supplementation as a preventive means in the general population is still debatable. In the CORONAVIT trial, there was no statistically significant difference in the incidence or severity of acute COVID-19 infection between the subjects treated with Vitamin D and those without supplementation for both intention-to-treat and sensitivity analysis.[31] In the recent Philippine COVID-19 clinical practice guidelines, there is also insufficient evidence based from randomized clinical trials in other countries, on the use of Vitamin D supplementation as an adjunct therapy.[32]

Limitations and recommendations

There were some limitations in this study. Not all the inflammatory parameters requested during the entire hospitalization were taken into account, including the dates of final results from the baseline values in the analysis. Medications that were used for treatment of COVID-19 may also had an effect in the clinical outcomes. Another limitation is the possibility of information bias as the investigators relied on electronic medical records and clinical notes written on charts to gather the necessary data. Due to its retrospective nature, some records had missing data hence studies with larger sample size are also recommended for higher statistical power. The analysis for composite poor outcome may also overestimate the risk due to the small sample size. Confounding bias was addressed using regression analysis, although there may be other factors that could have influenced poor outcome that was not measured in this study due to unavailability in medical charts (residual confounding). The external validity may not also be generalizable to the current population since characteristics of the subjects in this study may be different from other institutions, especially in government

hospitals. Yet these observations, together with the relative safety of Vitamin D supplementation, further support the need for prospective or well-designed intervention trials aimed at exploring whether Vitamin D supplementation and correction of Vitamin D deficiency might prevent the risk of morbidity and mortality in patients with COVID-19 infection.

CONCLUSION

The high occurrence of hypovitaminosis D in severe and critical COVID-19 patients implies a potential relation to poor prognosis. Overall, patients with deficient, but not insufficient Vitamin D level had 6 times higher odds of composite poor outcome than those with normal Vitamin D after adjusting for potential confounders (Crude OR = 5.18; adjusted OR = 6.3). The inverse association between serum 25(OH)D levels and composite poor outcome (ICU admission, in-hospital mortality and morbidity) observed in our retrospective study suggests that a lower Vitamin D status upon admission may be an independent risk factor for poor prognosis in COVID-19.

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

CRediT Author Statement
MKAT: Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **RA:** Conceptualization, Methodology, Validation, Writing – original draft preparation, Writing review and editing, Supervision; **KL:** Conceptualization, Methodology, Validation, Writing – original draft preparation, Writing review and editing, Supervision.

Author Disclosure
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SUPPLEMENTARY TABLES

Supplementary Table A. Screening for potential confounders: Univariable and multivariable logistic regression analysis of factors associated with Vitamin D level

	Crude OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*
Age (in years)	1.045 (1.012-1.078)	0.006*	1.044 (0.994-1.097)	0.084
Sex				
Female	Ref	Ref	Ref	Ref
Male	1.313 (0.589-2.929)	0.505	Removed	Removed
Weight (in kg)	0.982 (0.944-1.020)	0.344	Removed	Removed
BMI (in kg/m^2)	0.952 (0.866-1.047)	0.309	Removed	Removed
COVID-19 severity				
Mild	Ref	Ref	Ref	Ref
Moderate	3.202 (0.168-61.009)	0.439	4.092 (0.182-92.043)	0.375
Severe/ Critical	60.304 (3.252-1118.15)	0.006*	42.60 (1.835-988.65)	0.019*
Co-morbid condition, % with				
Hypertension	2.072 (0.901-4.768)	0.087	2.648 (0.748-9.381)	0.131
Diabetes Mellitus	0.500 (0.117-2.136)	0.349	Removed	Removed
Cerebrovascular Disease	2.550 (0.324-20.043)	0.373	Removed	Removed
Ischemic Heart Disease	3.611 (0.952-13.696)	0.059	6.428 (0.797-51.845)	0.081
Heart Failure	2.550 (0.324-20.043)	0.373	Removed	Removed
Chronic Kidney Disease (not on Dialysis/non-HD requiring)	2.763 (0.800-9.535)	0.108	Removed	Removed
Chronic Lung Disease (Asthma, COPD)	7.771 (0.364-166.956)	0.189	5.446 (0.146-203.83)	0.359
Thyroid Diseases	1.56 (0.488-4.986)	0.453	Removed	Removed
Chronic Liver Disease	1.494 (0.150-14.847)	0.732	Removed	Removed
Complete blood count, baseline				
WBC (x10^9)	1.050 (0.972-1.133)	0.216	Removed	Removed
Neutrophil count (%)	0.986 (0.953-1.021)	0.441	Removed	Removed
Lymphocyte count (%)	1.014 (0.981-1.049)	0.406	Removed	Removed
Hemoglobin (g/L)	1.003 (0.980-1.026)	0.814	Removed	Removed
Platelet (x10^9)	1.00 (0.995-1.002)	0.531	Removed	Removed
Electrolytes, baseline				
Sodium (mmol/L)	0.991 (0.937-1.048)	0.756	Removed	Removed
Potassium (mmol/L)	1.276 (0.605-2.692)	0.523	Removed	Removed
Creatinine (mmol/L)	1.008 (1.000-1.016)	0.064	Removed	Removed
Inflammatory markers, baseline				
Ferritin (ng/mL)	1.000 (1.000-1.000)	0.096	Removed	Removed
LDH ((U/L))	1.001 (1.000-1.002)	0.014*	1.001 (0.999-1.002)	0.437
D-dimer (ng/ml)	1.295 (1.029-1.629)	0.027*	1.058 (0.830-1.349)	0.648
Procalcitonin (ng/ml)	1.712 (0.978-2.997)	0.060	1.224 (0.442-3.384)	0.697
C-reactive Protein (mg/L)	1.037 (0.948-1.134)	0.428	Removed	Removed

Ref: Reference category; Variables wherein *p* of crude OR is in bold font were included in the multiple logistic regression analysis

Supplementary Table B. WHO Classification of COVID-19 based on severity

Mild Illness	Symptomatic patients presenting with fever, cough, fatigue, anorexia, myalgias; other non-specific symptoms such as sore throat, nasal congestion, headache, diarrhea, nausea and vomiting; loss of smell (anosmia) or loss of taste (ageusia) preceding the onset of respiratory symptoms with NO signs of pneumonia or hypoxia
Moderate Illness	Patients with clinical signs of non-severe pneumonia (e.g., fever, cough, dyspnea, respiratory rate (RR) = 21-30 breaths/minute, peripheral capillary oxygen saturation (SpO_2) >90% on room air)
Severe Illness	With clinical signs of severe pneumonia or severe acute respiratory infection as follows: fever, cough, dyspnea, RR>30 breaths/ minute, severe respiratory distress or SpO_2 <90% on room air
Critical Illness	Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction

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ORIGINAL ARTICLE

Critical Illness-Related Corticosteroid Insufficiency (CIRCI) Among Patients with COVID-19 at a Tertiary Hospital: Clinical Characteristics and Outcomes*

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Abstract

Objectives. Among critically ill patients, there is usually impairment of the hypothalamic-pituitary-adrenal axis, leading to a condition known as critical illness-related corticosteroid insufficiency (CIRCI). This investigation aims to determine the incidence of and characterize CIRCI among patients with COVID-19 as well as to analyze the outcomes of these critically ill patients.

Methodology. This is a single-center, retrospective cohort study that investigated the occurrence of CIRCI among critically ill patients infected with COVID-19.

Results. In this cohort, there were 145 COVID-19-positive patients with refractory shock, which reflects that 22.94% of the COVID-19 admissions have probable CIRCI.

Patients who were given corticosteroids were found to have statistically significant longer median days on a ventilator (p=0.001). However, those on the corticosteroid arm were at higher risk of morbidity and mortality and a greater proportion had organ dysfunction. Multivariable logistic regression analysis revealed that SOFA score was a significant predictor of mortality in CIRCI (p=0.013).

Conclusion. CIRCI has a unique presentation among patients with COVID-19 because of the presence of a high level of inflammation in this life-threatening infection. It is possibly a harbinger of a markedly increased risk of mortality in these patients.

Key words: adrenal insufficiency, COVID-19, critical illness, shock

INTRODUCTION

Towards the end of 2019, the world was struck by a pandemic in the form of a disease known as COVID-19, brought about by SARS-CoV-2, which originated in Wuhan, Hubei Province, China. SARS-CoV-2 belongs to the betacoronavirus 2B lineage, distinct from Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). Symptoms of COVID-19 infection include fever, cough, difficulty breathing, myalgia, fatigue, normal or low leukocyte counts or lymphopenia, and infiltrates on chest radiography indicative of pneumonia.[1] Indeed, COVID-19 is a serious global pandemic with a markedly high burden of disease.

In the Philippines, the total number of cases has reached 36,438, with 632 of these cases admitted at the Philippine General Hospital (PGH) at the time of this study's conclusion.[2] Severe cases of COVID-19 can present with organ dysfunction in the form of shock, acute respiratory distress syndrome, acute cardiac dysfunction, and acute kidney injury. In a cohort of 138 patients admitted at a hospital in Wuhan, as many as 26.1% of the patients were admitted to the intensive care unit (ICU) because of organ

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dysfunction.[1] Based on data from China, where the disease reportedly originated, about 13.2 to 21.3% of patients afflicted with COVID-19 had severe or fatal infections.[3] A significant number of patients afflicted with COVID-19 warrant intensive care. Septic shock occurred in about 4 to 8.7% of patients. The mortality is also high among critically ill patients reaching 61.7% at 28 days, with a median time from admission to death of 7 days.[4] On the other hand, international data showed that shock occurred in about 23 to 31% of patients in the ICU and that 70% of patients who succumbed to COVID-19 infection had septic shock.[1,5,6] Truly, a significant proportion of patients infected with COVID-19 present with critical illness in the form of septic shock or acute respiratory distress syndrome (ARDS), necessitating intensive care.

Among critically ill patients, there is usually impairment of the hypothalamic-pituitary-adrenal axis, leading to a condition known as critical illness-related corticosteroid insufficiency (CIRCI). This condition is characterized by a lack of corticosteroid response proportional to the level of stress. Patients afflicted with CIRCI usually present with refractory hypotension which may also be accompanied by hypoglycemia, electrolyte abnormalities, metabolic acidosis, and eosinophilia.[7] The incidence of CIRCI can be as high as 60% in patients with septic shock.[8] Analysis of different cohorts shows that the incidence of CIRCI among patients with sepsis ranged from 12% to as high as 75%.[9] Dysregulated systemic inflammation in the setting of CIRCI leads to organ dysfunction placing patients at high risk for prolonged vasopressor and ventilatory dependence and mortality. CIRCI is commonly found in a wide range of acute conditions such as sepsis, septic shock, severe community-acquired pneumonia, ARDS, cardiac arrest, head injury, trauma, burns, and after major surgery.[10] It has also been found that there is a twenty-fold higher incidence of symptomatic adrenal insufficiency in critically ill patients being managed in the ICU for more than two weeks,[11] which is a common scenario among patients afflicted with COVID-19. The diagnosis of CIRCI is established based on clinical findings of refractory hypotension unresponsive to fluid hydration, along with high or increasing requirements for vasopressors. According to the Society of Critical Care Medicine and European Society of Intensive Care Medicine, the diagnosis of CIRCI is confirmed through a random cortisol measurement of <10 mcg/dl or delta cortisol (change in baseline cortisol at 60 minutes of <9 mcg/dl) after cosyntropin or ACTH (250 mcg) administration.[12]

The cornerstone of the management of CIRCI is treatment with glucocorticoids. Various studies have demonstrated that the use of glucocorticoids at a stress dose (i.e., hydrocortisone at 200-300 mg/day) promoted hemodynamic function and improved survival of patients with septic shock by reducing organ system dysfunction, ventilatory support, and ICU days.[13] In terms of adverse effects, corticosteroids have been associated with hyperglycemia, myopathy, weight gain, easy bruisability, and osteopenia. However, these adverse effects are usually seen in patients with prolonged use of corticosteroids rather than in the critical care setting. A stress dose given over a short period - such as in refractory shock in critically ill patients is not expected to cause deleterious effects, such as immunosuppression. However, hyperglycemia and myopathy can still occur even with the short-term use of glucocorticoids.[14] Still, the current pool of evidence suggests that low-dose corticosteroids, i.e., hydrocortisone at 200-300 mg/day given for three days or more, contribute to improving survival and hemodynamic stability among critically ill patients without conferring a significant risk for adverse events.[12]

There is limited data on the use of glucocorticoids, which is the first-line treatment in CIRCI, in the setting of viral pneumonia. Several studies on patients with influenza A/H1N1 demonstrated the benefits of using glucocorticoids (methylprednisolone at 1 mg/kg/day or hydrocortisone at 300 mg/day) in patients with severe ARDS in terms of decreasing lung injury scores, multiple organ dysfunction scores, and mortality rate. However, other trials on patients afflicted with influenza A/H1N1 failed to detect any improvement in symptoms and even showed a trend towards an increase in mortality rate with glucocorticoid use.[13] In these trials though, patients in the glucocorticoid arm had a more severe illness which could possibly account for the increase in mortality rate observed in these cohorts.[15] For patients with SARS pneumonia, data from Guangzhou showed that 79.6% of patients who were given glucocorticoids had a lower mortality rate and shorter duration of hospitalization. However, it is worth noting that most trials on the use of glucocorticoids in patients with viral pneumonia were on patients with ARDS, rather than on those with septic shock. Therefore, further investigations on the benefit of glucocorticoids in patients with viral pneumonia and septic shock are indeed warranted.[13]

In cases of COVID-19, glucocorticoids were used for specific indications such as septic shock and ARDS. For instance, among the 138 admitted patients in a single center in Wuhan, as many as 44.9% received glucocorticoids. However, the outcomes of these patients were not fully analyzed.[1] In a cohort of 15 critically ill patients in Wuhan, the seven patients given glucocorticoids did not exhibit a survival benefit as they eventually expired. The study suggested that glucocorticoid treatment within the first 3 to 5 days of admission improved oxygen saturation and arterial oxygen tension/inspiratory oxygen fraction (PaO_2)/(FiO_2), thereby aiding in reducing organ dysfunction and shock. However, the small sample size of this cohort and the absence of a matched control group entail that such findings are interpreted with caution.[3] In another cohort of 201 patients at Jinyintan Hospital in Wuhan, it was shown that among patients with ARDS, methylprednisolone, which was given to 30.8% of the patients, reduced the risk of mortality (HR of 0.38, at 95% CI, 0.20-0.72). However, it was not specified how many of these patients suffering from ARDS were also in septic shock.[16] The recommendation for using dexamethasone for oxygen-requiring COVID-19 infection stems from the results of the Randomized

Evaluation of COVID-19 Therapy (RECOVERY) trial, which demonstrated that dexamethasone reduced 28-day mortality in these patients.[5] It can reduce mortality by up to a third.[17] There is an urgent need to investigate the development of CIRCI, its effect on clinical outcomes, and the response of COVID-19-positive patients to treatment with glucocorticoids.

Currently, there is a paucity of data on the incidence of CIRCI among COVID-19-positive patients, especially in the local setting. The extent to which CIRCI serves as a risk factor for poor outcomes among patients with COVID-19 and the effects of treatment with glucocorticoids on the clinical course of these patients are still crucial points of investigation. Extending the investigation of CIRCI to patients with COVID-19 will facilitate a deeper understanding of these two complex diseases. Addressing this knowledge gap will shape decision-making in the intensive care setting because CIRCI is a treatable condition. Glucocorticoid treatment when utilized in the appropriate context is potentially lifesaving. A better grasp of CIRCI in the context of COVID-19 infection is pivotal in improving the quality of intensive care management for COVID-19-positive patients.

This study had the following objectives: to describe the incidence of and characterize probable and definite CIRCI among patients with COVID-19; to determine the clinical outcomes (morbidity, mortality, ventilator days, number of days in shock, vasopressor dependent days, ICU days, length of hospitalization, recovery rate) among critically ill COVID-19-positive patients in shock who were given glucocorticoids and those who were not given glucocorticoids, and to detect the incidence of adverse events in those patients who were given glucocorticoids.

METHODOLOGY

Study design

This was a single-center, retrospective cohort study that investigated the occurrence of CIRCI among critically ill patients infected with COVID-19. A review of records among admitted patients was done.

Study duration

The retrospective chart review included patients admitted at the designated COVID-19 inpatient areas of the Philippine General Hospital from March 31, 2020 (which coincides with the assignment of the tertiary hospital as a COVID referral center) until June 30, 2020, thereby covering a total period of three months.

Inclusion criteria

All patients aged 19 years old and above, with confirmed COVID-19 infection documented on real-time reverse transcription-polymerase chain reaction (rRT-PCR) assay, with an admitting diagnosis of shock or developed refractory hypotension during the admission (i.e., requiring at least 0.5 mcg/kg/min of norepinephrine or its equivalent dose with another vasopressor or with increasing vasopressor requirement) were included in the analysis. Refractory hypotension or shock was defined as systolic blood pressure of persistently <90 mmHg after adequate fluid resuscitation for at least 30 minutes in the presence of hypovolemia; need for vasopressors to maintain adequate organ perfusion; and signs of hypoperfusion such as tachycardia, altered mental status, confusion or encephalopathy, cold extremities, oliguria, and blood lactate >2 mmol/L.[18]

In this study, the case definitions used for CIRCI were the following: A probable case of CIRCI is a patient who exhibits clinical manifestations of CIRCI such as refractory hypotension responding poorly to fluid resuscitation and vasopressors or increasing vasopressor requirements, which may or may not be accompanied by other symptoms and signs of adrenal insufficiency such as weakness, fatigue, loss of appetite, abdominal pain, nausea, vomiting, hypoglycemia, hyperkalemia, hyponatremia, metabolic acidosis, and eosinophilia but without a random cortisol laboratory result to document hypocortisolism during the period of critical illness. A definite case of CIRCI is a patient exhibiting clinical findings of CIRCI as mentioned previously, with random serum cortisol of <10 mcg/dl or delta cortisol of <9 mcg/dl at 60 minutes after ACTH stimulation testing, which establishes an inadequate corticosteroid response for the level of stress. The ACTH stimulation test was done for patients with indeterminate baseline serum cortisol levels between 11-34 mcg/dl.[10] Serum cortisol levels were measured using the cortisol (125I) RIA kit (Ref: RK-240CT). The ACTH stimulation test was performed using Synacthen (tetracosactide acetate), which contains 250 mcg per ampule. Analytical validity standards were met. The conversion factor used to express serum cortisol levels from the unit nmol/L to mcg/dl was 1 mcg/dl = 27.59 nmol/L.[19]

The case definition for a COVID-19 confirmed case is based on the WHO Global Surveillance for Disease Interim Guidance.[20] A COVID-19 confirmed case is a patient with or without symptoms attributed to COVID-19, with documented SARS-CoV-2 infection. The laboratory test used to confirm the presence of COVID-19 infection is the rRT-PCR assay, where in SARS CoV-2 can be detected in nasal or pharyngeal samples, sputum, bronchoalveolar lavage fluid, and other bodily fluids.[4]

Exclusion criteria

The patients who were admitted as COVID-19 suspect cases but subsequently found to be negative on rRT-PCR COVID-19 testing and diagnosed as not afflicted with COVID-19 by the Infectious Disease service were excluded from the final analysis. Patients who were weaned off vasopressors immediately (within 4 hours from onset of shock) upon additional fluid resuscitation or loading of

antibiotics were excluded from the analysis because such patients are unlikely to have developed CIRCI. Other exclusion criteria were the use of systemic glucocorticoids in the form of at least 40 mg of prednisolone or equivalent per day for more than one week before admission for COVID-19, or the use of etomidate, ketoconazole, and other agents known to cause adrenal insufficiency.[21] Patients with a history of adrenal disease or adrenalectomy, pituitary surgery, or pituitary irradiation and pregnant individuals were also excluded from the analysis.

Outcomes

Relevant clinical characteristics such as the median age, proportion of males and females, median blood pressure, the top etiologies of shock, vasopressor dose, number of days on vasopressors, ventilator days, length of ICU stay, length of hospital stay and morbidity and mortality rates of patients with refractory shock and probable CIRCI were described. Laboratory values such as the serum cortisol levels of the subjects were also examined. The rate of corticosteroid utilization and the type and dose of corticosteroid administered were also obtained.

An in-depth analysis of the key clinical outcomes of the patients in refractory shock who were started on corticosteroids and those who were not started on corticosteroids was made. Outcomes included the number of days on vasopressors, ventilator days, vasopressor requirement, length of ICU stay, length of hospital stay, morbidity and mortality rate, and the ICU severity of illness score in the form of the Mortality Probability Model (MPM).

For the patients in the steroid group, a comparison of the clinical outcomes was made between patients given hydrocortisone and those who were given other types of corticosteroids such as dexamethasone, prednisone and methylprednisolone. The same clinical outcomes were examined for the patients who were started on different doses of hydrocortisone: at exactly 200 mg/day which is the recommended dose for patients with CIRCI,[10] at <200 mg/day, and >200 mg/day.

Statistical methods

The analysis of the data obtained from this retrospective chart review was performed using Stata Version 15.1. In determining the baseline characteristics of patients with CIRCI, the median and range were used as summary measures because almost all quantitative variables were not normally distributed. The distribution was tested using the Shapiro-Wilk test of normality. Qualitative variables were reported using count and proportion or rate. In making comparisons between the groups started on corticosteroids and those who were not started on corticosteroids, and between the hydrocortisone group and the non-hydrocortisone group, the Mann-Whitney U test of difference between medians of two groups, and the Z test of two proportions were employed.

The groups utilizing various doses of hydrocortisone were analyzed using the Kruskal-Wallis test of difference between medians of more than two groups and the Chi-square test of homogeneity (proportion) of more than two groups. The level of significance utilized for the statistical tests employed in this study was $\alpha = 5\%$.

Multiple logistic regression analysis was done to determine the predictors of mortality among patients with CIRCI infected with COVID-19. After conducting a literature review, the authors selected the following variables—severity of illness score, etiology of shock, timing of initiation of steroids relative to the number of days in shock, presence of hypoglycemia, and duration of steroid use—as co-variates or predictor variables in the regression analysis based on previous evidence demonstrating that such factors affect clinical outcomes in the setting of COVID-19 infection.[16,18] All these variables were included in the multivariate logistic regression model because these were hypothesized to be risk factors for increased mortality in CIRCI and were of interest to the authors.

Ethical issues

This research focusing on patients with COVID-19 was a sub-study of the mixed methods research project entitled, "The Development and Pilot Testing of a Protocol for the Initiation and Use of Corticosteroids for Critical Illness-Related Corticosteroid Insufficiency for Patients Admitted with Shock at the Philippine General Hospital," approved by the University of the Philippines Manila Research Ethics Review Board, with the registration number 2020-297-01.

RESULTS

Study population characteristics

In this cohort, there were 145 patients with COVID-19 included in the final analysis. This corresponds to 22.94% of all the COVID-19-positive admissions at PGH (N= 632) during the study period, presenting with refractory shock, meeting the criteria for probable CIRCI. Twenty-two patients had available serum cortisol results. Thirteen of these patients were in the steroid group, with a median baseline cortisol level of 25.4 mcg/dl, and nine of these patients were in the non-steroid group, with a median baseline cortisol level of 25.08 mcg/dl. Four patients met the criteria for definite CIRCI based on initial serum cortisol results or ACTH stimulation testing. Two patients with indeterminate cortisol results underwent ACTH stimulation testing to ascertain the presence of CIRCI.

The median age of the patients was 63 years old and the majority were males (57.24%). Septic shock was the etiology of shock for 72.22% of the population. The average lactate level of the patients was 2.887 mmol/L, which is consistent with the presence of vasopressor-dependent shock.[22] Subjects were vasopressor dependent mostly on norepinephrine, with some patients requiring dopamine,

epinephrine, or dobutamine in addition to norepinephrine, for a median of 2 days with a range of 0 to 49 days. The median Sequential Organ Failure Assessment (SOFA) score was 13 which suggests that most of the patients included had a high mortality rate with a 40-50% risk of death.[23] Other ICU risk prognostic scores echoed this intensified risk. Majority of the patients suffered from acute respiratory failure, with 85.42% requiring a ventilator, and as much as 81.12% diagnosed with ARDS. There were also high rates of multiple organ dysfunction, with acute kidney injury in 67.59% of the subjects and central nervous system dysfunction in 79.17% of patients. The mortality rate of this cohort of patients was high at 90.34% (Table 1).

In terms of laboratory parameters, the median random cortisol level of the entire study population was 25.26 mcg/dl. This level is still way below the threshold of 34 mcg/dl and above which CIRCI is unlikely.[10] This strengthens the finding of CIRCI in this cohort of patients. For the patients with indeterminate cortisol level results who underwent ACTH stimulation testing, 50% had an increase in serum cortisol from baseline of more than 9 mcg/dl, while 50% did not exhibit an elevation in cortisol levels.

The patients were also screened for the presence of laboratory findings suggestive of adrenal insufficiency. Hyponatremia, i.e., a sodium level below 135 mEq/L, was present in 26.9% of the subjects in this cohort. On the other hand, hyperkalemia exemplified by a potassium value greater than 5.5 mEq/L, was seen in 16.55% of the participants. Hypoglycemia, defined as a capillary blood glucose level of less than 70 mg/dl, was found in 8.97% of the patients. Metabolic acidosis developed in 11.11%, and eosinophilia found in 2.10% of patients (Table 1).

Table 1. Baseline characteristics and outcomes of COVID-19 patients with CIRCI

Age, median (range)	63 (19-95)
Sex, count (percent)	
Males	83 (57.24%)
Females	62 (42.76%)
Top 3 etiologies of shock, count (percent)	
Septic	104 (72.22%)
Multifactorial	23 (15.97%)
Cardiogenic	9 (6.25%)
SOFA score, median (range)	13 (1-20)
APACHE II score, median (range)	29 (7-52)
MPM score, median (range)	82.7 (14.8-98.6)
On ventilator, count (percent)	123 (85.42%)
With ARDS, count (percent)	116 (81.12%)
With acute kidney injury, count (percent)	98 (67.59%)
With oliguria, count (percent)	36 (25.90%)
With CNS dysfunction (Glasgow Coma Scale of <15), count (percent)	114 (79.17%)
With hypoglycemia (CBG <70 mg/dl), count (percent)	13 (8.97%)
With metabolic acidosis, count (percent)	16 (11.11%)
With eosinophilia, count (percent)	3 (2.10%)
Baseline cortisol level (nmol/L), median (range)	696.06 (22-1668)
Dose of vasopressors (mcg/kg/min), median (range)	
On 1 vasopressor	0.5 (0.09-10)
On 2 vasopressors	0.4 (0.2-15)
On 3 vasopressors	10 (0.3-10)
Number of days on vasopressors, median (range)	2 (0-49)
Number of days on ventilator, median (range)	4 (0-76)
Length of ICU stay, median (range)	5 (0-56)
Length of entire hospital stay, median (range)	9 (1-81)
Morbidity, count (rate)	126 (86.90%)
Mortality, count (rate)	131 (90.34%)

Table 2. Use of corticosteroids for COVID-19 Patients with CIRCI

Corticosteroid Use, count (rate)	102 (70.83%)
Hydrocortisone	32 (31.37%)
<200 mg/day	3 (9.38%)
200 mg/day	16 (50.00%)
>200 mg/day	13 (40.62%)
Non-hydrocortisone	70 (68.63%)
Number of days in shock when corticosteroids were Initiated, median (range)	0 (0-12)
Days on corticosteroids, median (range)	4 (1-41)
Blood pressure after corticosteroids were initiated, count (percent)	
Improved	93 (70.45%)
Still hypotensive	39 (29.55%)
Dose of vasopressors (mcg/kg/min) after corticosteroids were initiated, median (range)	0.35 (0-7.8)

Use of corticosteroids for COVID-19 patients with CIRCI

For COVID-19 patients with refractory shock, there was a high rate of utilization of steroids at 70.83%. Half the population was given hydrocortisone at 200 mg/day, which is the recommended therapeutic regimen for CIRCI. As many as 68.63% were given steroids other than hydrocortisone, mostly as dexamethasone (71.57% of the non-steroid group). One patient received methylprednisolone. Corticosteroids were initiated after a median of less than one day in shock, with a range of 0 to 31 days. The duration of corticosteroid administration lasted for a median of 4 days. After corticosteroids were initiated, blood pressure improved in 70.45% of the patients (Table 2).

Use of corticosteroids and patient outcomes

Patients who were given corticosteroids were found to have statistically significant longer median days on a ventilator (p=0.001). However, those on the corticosteroid arm were at higher risk of morbidity and mortality as signified by statistically significant higher APACHE II scores (p=0.0233), MPM scores (p=0.006), and a greater proportion of patients with acute kidney injury (p=0.028), oliguria,(p=0.020) and CNS dysfunction (p=0.019). There were no significant differences between the length of hospital stay, morbidity, and mortality rates between the steroid and non-steroid groups. (Table 3).

Two out of the three patients with a baseline serum cortisol level of less than 10 mcg/dl died. Ten out of the fourteen patients with indeterminate serum cortisol levels, i.e., between 11-34 mcg/dl expired during the study. For the two patients who underwent ACTH stimulation testing, the non-responder died, and the responder who exhibited a greater than 9 mcg/dl increase in serum cortisol after ACTH administration, survived and was discharged.

There were no significant differences in morbidity (78.12% in the hydrocortisone group vs. 91.43% in the non-hydrocortisone group, p=0.062) and mortality (87.50% in the hydrocortisone group vs. 92.86% in the non-hydrocortisone group, p=0.376) rates among patients given hydrocortisone and those who received other types of steroids, albeit patients in the hydrocortisone arm had a slightly longer median length of hospital stay (13 days vs. 7.5 days, p=0.0231). This could likely be attributed to the greater severity of shock present in those on the hydrocortisone group (Table 5). Likewise, no significant differences in morbidity (p=0.279) and mortality (p=0.125) rates were detected among groups on varying doses of hydrocortisone (Appendix).

In terms of adverse events associated with corticosteroid use, steroid-induced hyperglycemia ensued in only 7 patients (6.8%) in the corticosteroid arm. One patient had documented hypernatremia, or a sodium level of more than 145 mEq/L after corticosteroid was initiated. There were no cases of steroid-induced myopathy, secondary infection, or bleeding detected in this cohort of patients. Overall, there was a low incidence of adverse events associated with corticosteroid use.

Predictors of mortality among COVID-19 patients with CIRCI

On univariable analysis, higher ICU risk prognostic scores, in terms of the SOFA (OR=1.31, CI 1.06 to 1.62, p=0.013 for the multivariable analysis and OR=1.37, CI 1.15 to 1.63, p=<0.001 for the univariable analysis) and MPM score (OR=1.03, CI 1.01 to 1.06, p=0.002) predict mortality among COVID-19 patients with CIRCI (Table 4). For every 1% increase in the SOFA score, the odds of mortality increase by 31%. The number of days in shock was also a significant predictor of mortality in this cohort of patients. For each day in shock since the steroid was initiated, the odds of mortality decreased by 18% as demonstrated in the unadjusted model. Logistic regression analysis also demonstrated that the duration of steroid use had an associated effect on mortality among patients with CIRCI and COVID-19.

Table 3. Comparison of clinical characteristics and outcomes of patients started on corticosteroids and patients not given corticosteroids

	With use of steroids	Without use of steroids	*p*
SOFA score, median (range)	13 (3-20)	12 (1-18)	0.1668
APACHE II score, median (range)	30 (11-52)	26 (7-41)	0.0233
MPM score, median (range)	89.45 (14.8-98.6)	66.25 (21.5-97.5)	0.0006
On ventilator, count (percent)	90 (88.24%)	33 (80.49%)	0.227
With ARDS, count (percent)	80 (80.00%)	35 (83.33%)	0.644
With acute kidney injury, count (percent)	75 (73.53%)	23 (54.76%)	0.028
With oliguria, count (percent)	31 (31.63%)	5 (12.50%)	0.020
With CNS dysfunction (Glasgow coma scale <5), count (percent)	85 (84.16%)	28 (66.67%)	0.019
With hypoglycemia (CBG <70 mg/dl), count (percent)	8 (7.84%)	5 (11.90%)	0.524
Baseline cortisol Level (nmol/L), median (range)	702.1 (22-1668)	691.92 (347.27-1427.2)	0.8673
Number of days on vasopressors, median (range)	3 (0-37)	2 (0-49)	0.4214
Number of days on ventilator, median (range)	5 (0-76)	2 (1-50)	0.0001
Highest vasopressor requirement, median (range)			
Vasopressor 1	0.5 (0.2-10)	0.415 (0.09-10)	0.5454
Vasopressor 2	0.4 (0.2-15)	1 (0.3-10)	0.4485
Vasopressor 3	10 (0.3-10)	-	-
Length of ICU stay, median (range)	6 (0-56)	3 (0-41)	0.0547
Length of entire hospital stay, median (range)	9 (1-76)	9.5 (2-81)	0.7050
Morbidity, count (rate)	89 (87.25%)	37 (88.10%)	0.890
Mortality, count (rate)	93 (91.18%)	37 (88.10%)	0.571

Table 4. Multiple logistic regression analysis of predictors of mortality among COVID-19 positive patients with CIRCI

Factors	Univariable			Multivariable		
	OR	95% CI	*p*	OR	95% CI	*p*
Steroid use	1.40	[0.44, 4.44]	0.572	0.39	[0.08, 1.99]	0.258
MPM score	1.03	[1.01, 1.06]	0.002	1.02	[0.99, 1.06]	0.111
SOFA score	1.37	[1.15, 1.63]	<0.001	1.31	[1.06, 1.62]	0.013
APACHE II score	1.13	[1.05, 1.22]	0.001	1.03	[0.94, 1.14]	0.476
Etiology of shock						
Septic	Reference			Reference		
Cardiogenic	0.25	[0.04, 1.45]	0.123	2.16	[0.16, 29.35]	0.563
Hypovolemic	0.07	[0.01, 0.43]	0.004	0.05	[0.004, 0.51]	0.012
Multifactorial	0.76	[0.15, 3.91]	0.740	2.05	[0.27, 15.24]	0.484
Days in shock when steroids were started	0.82	[0.68, 0.99]	0.041	-		
Days on steroids	0.94	[0.85, 1.05]	0.274	-		
Hypoglycemia	0.55	[0.11, 2.78]	0.469	0.65	[0.003, 1.90]	0.118

Table 5. Comparison of patient characteristics and outcomes between patients given hydrocortisone and patients given other forms of steroids

	Hydrocortisone	Non-Hydrocortisone	*p*
SOFA score, median (range)	12 (3-18)	14 (5-20)	0.0847
APACHE II score, median (range)	31.5 (11-47)	30 (14-52)	0.8551
MPM score, median (range)	90 (14.8-98.6)	89.2 (21.6-98.5)	0.9196
On ventilator, count (percent)	25 (78.12%)	65 (92.86%)	0.032
With ARDS, count (percent)	21 (65.62%)	59 (86.76%)	0.014
With acute kidney injury, count (percent)	24 (75.00%)	51 (72.86%)	0.820
With oliguria, count (percent)	8 (25.81%)	23 (34.33%)	0.399
With CNS dysfunction (Glasgow coma scale <15), count (percent)	26 (81.25%)	59 (85.51%)	0.586
With hypoglycemia (with CBG <70 mg/dl), count (percent)	4 (12.50%)	4 (5.71%)	0.237
Baseline cortisol level (nmol/L), median (range)	655.05 (34-1668)	957.13 (22-1349.4)	0.7697
Number of days on vasopressors, median (range)	3 (0-37)	4 (0-32)	0.4817
Number of days on ventilator, median (range)	4.5 (0-76)	6 (1-26)	0.9683
Highest vasopressor requirement, median (range)			
Vasopressor 1	0.5 (0.2-10)	0.445 (0.2-2)	0.1456
Vasopressor 2	0.575 (0.3-10)	0.4 (0.2-15)	0.3902
Vasopressor 3	5.15 (0.3-10)	10 (10-10)	0.1573
Length of ICU stay, median (range)	6 (0-56)	5 (0-36)	0.5669
Length of entire hospital stay, median (range)	13 (1-76)	7.5 (1-51)	0.0231
Morbidity, count (rate)	25 (78.12%)	64 (91.43%)	0.062
Mortality, count (rate)	28 (87.50%)	65 (92.86%)	0.376

DISCUSSION

COVID-19 is a systemic disease with a complex form of management. Most patients with severe and critical COVID-19 infection present with multiple organ dysfunction. Shock is also prevalent among patients with COVID-19, with a significant proportion dependent on vasopressors, reaching 22 to 67% according to several studies.[24] In this cohort of patients at the PGH, a notable proportion of 22.94% of COVID-19 admissions qualify as probable CIRCI, thus representing a serious disease burden.

This population cohort consisted of individuals with a high risk for morbidity and mortality because of the presence of critical COVID-19. Several reliable ICU prognostic scores, namely MPM and APACHE II scores, validated that this cohort had a high risk for adverse outcomes. Such an inherent characteristic of this population accounts for the mostly poor outcomes seen in this population. COVID-19-positive patients with refractory shock who were started on steroids were found to have statistically significant longer days on a ventilator. This is likely because most of the patients on the steroid arm also had ARDS, thus entailing a longer period of ventilatory support. There were also statistically significant higher rates of baseline acute kidney injury, oliguria, and CNS dysfunction among patients in the steroid group. The presence of multiple organ dysfunction is a significant barrier to weaning off the ventilator, thus accounting for the longer time of dependence on the ventilator for patients in this arm.

Overall, patients in both groups did not exhibit statistically significant differences in morbidity and mortality rates, though the logistic regression analysis suggested an association between a longer duration of steroid treatment and a decrease in mortality for COVID-19 patients with refractory shock. The power achieved on the analysis of steroid use as a predictor variable (OR = 0.39) in a multivariable regression with other co-variates (Pseudo-R2 = 0.3140) and two-tailed alpha=5% level of significance is 77%. Given that power should ideally be at 80%, the study may have not established sufficient evidence that steroid use is a significant predictor of mortality because it falls short of a few more samples. The relatively small sample size of this cohort could have affected the detection of a stark contrast in terms of morbidity and mortality between the two groups.

In this study which was done during the early days of the pandemic, the patients received the type of treatment appropriate for the presentation of their COVID-19 infection. Some of the patients had refractory shock but no hypoxemia and predominantly exhibited weakness, fatigue, or gastrointestinal symptoms instead. Yet, a substantial number of the patients with refractory shock also presented with acute respiratory failure (85.42%); thus, dexamethasone was widely utilized because it is the recommended treatment for oxygen-requiring severe and critical COVID-19 infection.[25] One patient was given methylprednisolone for acute respiratory distress syndrome. However, compared to hydrocortisone, dexamethasone and methylprednisolone do not have significant mineralocorticoid activity, and this phenomenon could explain why some patients remained in shock even after corticosteroids were initiated, thus accounting for the lack of statistically significant reduction in mortality seen in this cohort. Moreover, in contrast to the patients in the RECOVERY trial who were treated with corticosteroids for a median of 7 days, the patients in this study were given corticosteroids for a median of 4 days. The relatively shorter duration of treatment brought about by the high rates of early demise from critical COVID-19 infection may have also contributed to the lack of reduction in mortality seen in this cohort.

The results of this study done in the Philippines are also consistent with the trends found in other countries like China. The study of Yiming et al. also had a population similar to the Philippine cohort with most of the subjects receiving non-hydrocortisone steroid therapy and with high rates of organ dysfunction among the non-survivors.[26]

The presentation of CIRCI among patients with COVID-19 is unique. Individuals present with refractory shock, but the cortisol levels may be higher than those patients without COVID-19 infection because of the intense level of inflammation present in COVID-19 infection.[27] In this cohort of COVID-19-positive patients, the median cortisol level was at 25.26 mcg/dl compared to 24.15 mcg/dl for the non-COVID subgroup in the same institution.[28] However, a notable factor that ought to be considered is that not all patients in this cohort had random serum cortisol results because, in most of the patients, CIRCI was diagnosed based on the presence of refractory shock and subsequent therapeutic response to corticosteroids. The small sample size of patients with cortisol results could have served as a barrier in detecting significantly low cortisol levels in this cohort. Still, cortisol levels relatively higher than those seen in patients without COVID-19 infection were also observed in other studies.

In a case report of a 69-year-old male admitted for critical COVID-19, the median total cortisol level was 12 mcg/dl,[29] which was higher than the usual cortisol level of less than 10 mcg/dl for those with definite CIRCI.[10] High cortisol levels can be associated with increased mortality as well, in the setting of a dysregulated inflammatory response amidst the cytokine storm.[27,30] The ongoing massive systemic inflammation activates the stress response, accounting for the increase in cortisol levels that is somehow still seen. Therefore, the diagnosis of CIRCI is not dependent on cortisol levels alone. Across studies, the levels of cortisol among COVID-19-positive patients with CIRCI are varied. Interindividual variability when it comes to fluctuations in cortisol levels in response to stress may also account for this phenomenon.[27] CIRCI is more reliably determined by the inability of the cortisol level to address the extensive inflammation and metabolic demand.[31] Even if elevations of cortisol levels are seen, the action of cortisol is still insufficient to maintain hemodynamic stability during critical illness which can be due to the presence of tissue resistance to glucocorticoids.[24] Amidst the limitations of utilizing cortisol levels in diagnosing CIRCI, the determination of random serum cortisol levels is still valuable in guiding the course of therapy and future management strategies.

The pathophysiologic mechanisms surrounding the complex phenomenon of CIRCI in COVID-19 infection are multi-faceted. During critical illness in the context of COVID-19 infection, despite the increase in cortisol levels seen in some cases, this response is still not enough to meet the high demand for cortisol. Extremely high levels of inflammatory makers such as interleukins 1 and 6, and tumor necrosis factor α (TNF-α) disrupt the hypothalamic-pituitary-adrenal (HPA) axis. The release of ACTH stimulated by corticotrophin-releasing hormone (CRH) is blunted by TNF-α, thereby inhibiting the action of ACTH and angiotensin 2 on adrenal cells.[32-34] Therefore, the cytokine storm itself inhibits the hypothalamic-pituitary-adrenal axis. Another plausible mechanism is the production of amino acid sequences mimicking ACTH by SARS viruses, thereby resulting in the generation of antibodies that ultimately lead to central adrenal insufficiency.[35]

Indeed, the presence of CIRCI among COVID-19-positive patients is associated with significant morbidity and mortality. Thus, consensus-building on the optimal management strategies for COVID-19 patients with refractory shock is paramount, especially in light of the variability seen in diagnosing and treating CIRCI currently. Ideally, the use of hydrocortisone for COVID-19 infected patients with refractory shock should be incorporated into management protocols of institutions, as it is the corticosteroid with significant mineralocorticoid action that could address the hypotension. Currently, there is strong evidence suggesting that the use of hydrocortisone would produce the same extent of benefit as dexamethasone and methylprednisolone in reducing inflammation amidst respiratory damage, as well as mortality because of a class effect.[36,37] Hydrocortisone has also been shown to protect the endothelium against damage in the presence of severe inflammation.[35] The Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP) trial demonstrated that hydrocortisone at a dose of 200 mg per day, comes with an 80% probability of benefit.[38] Likewise, Liu et al., found that hydrocortisone can reduce mortality at 28 days even for patients suffering from ARDS, thus reinforcing the postulation of a class effect.[39]

The use of hydrocortisone for COVID-19-positive patients experiencing shock is consistent with the recommendations of several expert bodies, such as the Surviving Sepsis Guidelines for COVID-19,[40] the China National Commission[39] and the Philippine Living Clinical Practice Guidelines for COVID-19.[25] Even amidst the COVID-19 infection, corticosteroid administration has been deemed safe because it does not significantly affect the rates of secondary infection[41] nor the rates of clearance of the SARS-CoV-2 virus.[26]

This study performed at a tertiary referral center for COVID-19-positive patients was able to characterize CIRCI in the setting of COVID-19 infection. However, a significant limitation of this study is the small number of patients who had cortisol level determinations and subsequent ACTH stimulation testing due to constraints in resources in the local setting. Also, the impact of steroid use on mortality was not fully demonstrated because of the relatively small sample size. The study was also conducted at a time when the protocol on CIRCI was newly launched and was still undergoing pilot testing at the tertiary hospital, accounting for the gap in the consistent performance of cortisol testing

for patients suspected to have CIRCI. Thus, the findings from this research underscore the need to incorporate the diagnosis and management of CIRCI in critically ill COVID-19-positive patients in institutional protocols to guide clinical practice, especially since early detection and treatment of the condition can be lifesaving.

CONCLUSION

Among COVID-19-positive patients, CIRCI has a likely substantial disease burden. CIRCI has a unique presentation among COVID-19 patients because of the presence of a high level of inflammation in this life-threatening infection. It is possibly a harbinger of increased risk of poor clinical outcomes and mortality.

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Statement of Authorship
All authors certified fulfillment of the ICJME authorship criteria.

CRediT Author Statement
AEA: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration, Funding acquisition; **KWL:** Validation, Formal analysis, Investigation, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization; **MA:** Validation, Formal analysis, Investigation, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization; **CJ:** Conceptualization, Methodology, Software, Validation, Formal analysis, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision.

Author Disclosure
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APPENDIX

Appendix. Comparison of patient characteristics and outcomes of patients given different doses of hydrocortisone

	Hydrocortisone <200 mg/day	Hydrocortisone 200 mg/day	Hydrocortisone >200 mg/day	*p*
SOFA score, median (range)	16 (9-18)	11 (8-18)	12 (3-18)	0.5851
APACHE II score, median (range)	29 (19-47)	29.5 (15-40)	35 (11-47)	0.5102
MPM ccore, median (range)	52.2 (37.2-97.2)	92.5 (14.8-98.6)	78 (17.3-98.1)	0.6954
On ventilator, count (percent)	2 (66.67%)	11 (68.75%)	12 (92.31%)	0.279
With ARDS, count (percent)	2 (66.67%)	8 (50.00%)	11 (84.62%)	0.153
With acute kidney injury, count (percent)	3 (100%)	11 (68.75%)	10 (76.92%)	0.728
With Oliguria, count (percent)	-	3 (18.75%)	5 (38.46%)	0.443
With CNS dysfunction (Glasgow coma scale <15), count (percent)	2 (66.67%)	13 (81.25%)	11 (84.62%)	0.667
With hypoglycemia (CBG <70 mg/dl), count (percent)	-	3 (18.75%)	1 (7.69%)	0.740
Baseline cortisol level (nmol/L), median (range)	784.22 (784.22-784.22)	655.05 (311.5-1668)	34 (34-34)	0.2826
Number of days on vasopressors, median (range)	2 (1-19)	5.5 (0-26)	1 (0-32)	0.1316
Number of days on ventilator, median (range)	14 (4-24)	14 (2-76)	3 (0-42)	0.0603
Highest vasopressor requirement, median (range)				
Vasopressor 1	0.4 (0.3-2)	0.52 (0.2-10)	0.5 (0.2-3)	0.9711
Vasopressor 2	10 (10-10)	0.35 (0.3-2.2)	0.75 (0.3-10)	0.1649
Vasopressor 3	0.3 (0.3-0.3)	-	10 (10-10)	0.3173
Length of ICU stay, median (range)	14.5 (4-25)	6 (0-56)	2 (1-41)	0.3432
Length of entire hospital stay, median (range)	30 (3-53)	21 (5-76)	6 (1-43)	0.0112
Morbidity, count (rate)	2 (66.67%)	11 (68.75%)	12 (92.31%)	0.279
Mortality, count (rate)	2 (66.67%)	13 (81.25%)	13 (100%)	0.125

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REVIEW ARTICLE

Incidence, Recurrence and Mortality Among Filipinos With Differentiated Thyroid Cancer: A Systematic Review

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Abstract

Background. The majority of thyroid malignancies are differentiated thyroid carcinomas (DTCs). We examined the incidence, disease extent, recurrence and disease-specific mortality (DSM) of DTC among Filipinos residing in the Philippines and Filipino immigrants.

Methodology. In accordance with the 2020 PRISMA statement, we performed a systematic literature search in MEDLINE, Google Scholar, EBSCO, Cochrane and Clinicaltrials.gov for the period January 1, 1980 until January 27, 2022. Pooled incidence rate ratio and pooled proportions of disease extent, recurrence and DSM were determined.

Results. Literature search yielded 1,852 studies. Out of 26 articles retrieved, nine retrospective case controls and cohorts were included. Incidence of DTC was significantly higher in female Filipino immigrants compared with non-Hispanic whites (NHW). Distant metastases and recurrence were more common among Filipinos and Filipino immigrants compared with NHW. Limited data showed higher DSM in Filipino immigrants and NHW than Filipinos, which may be influenced by reporting bias.

Conclusions. This review supports the trend of increased incidence and recurrence of DTC among Filipinos, although case registries are essential to confirm these findings. In the setting of the newly released Philippine guidelines for DTC, prospective studies with active long-term follow-up will help detect any changes in the outcomes of DTC among Filipinos.

Key words: Filipino, thyroid malignancy, differentiated thyroid carcinoma, papillary thyroid carcinoma, follicular thyroid carcinoma

INTRODUCTION

Thyroid cancer is the most common endocrine malignancy worldwide. Based on the Philippine 2020 Cancer Registry and Research Annual Report, it is the ninth most common malignancy.[1] The majority of thyroid malignancies are differentiated thyroid carcinomas (DTC), specifically papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), which have an indolent course, low risk of recurrence after treatment and favorable prognosis.[2]

Data on the incidence and prognosis of DTC in the Philippines are mainly derived from tertiary centers. The Philippine General Hospital, the largest referral hospital in the country, reported that PTC in Filipinos have almost twofold larger tumor size at presentation, two to seven times more frequent distant metastases at diagnosis, and two- to threefold greater recurrence compared with the Koreans and Japanese.[3]

The global trend of immigration has led to an increasing number of Filipinos all over the world. The Surveillance, Epidemiology and End Results (SEER) database comprises of data from Atlanta, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Utah, New Jersey, Seattle-Puget Sound, San Francisco/Oakland, San Jose/Monterey, Los Angeles and all remaining areas of California, representing 54% of the Asian and Pacific Islander population in the US.[4] A review of the SEER database from 1973 to 1981 was the first to report that Filipino men and women have higher incidence rates of thyroid cancer. The average annual incidence of thyroid cancer was 7.3 per 100,000 for Filipino males and 17.3 per 100,000 for Filipino females, compared with 2.3 per 100,000 for Caucasian males and 5.4 per 100,000 for Caucasian females.[5] Similarly high incidence rates were noted in patients of Japanese and Hawaiian descent. Since then, multiple groups have investigated the effect of ethnicity, place of birth and immigration on the incidence, risk of recurrence and disease-specific mortality (DSM) of thyroid malignancy.

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Evidence-based management of DTC is guided by the 2015 American Thyroid Association (ATA) Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer. The latest recommendations discourage a single approach for all patients with DTC. Instead, treatment options and goals should be based on individual pre-operative characteristics and post-operative risk stratification.[6] Due to the increased incidence and higher risk for metastases and recurrence in Filipinos, some experts have deviated from the ATA recommendations and opt to have thyroidectomy done for thyroid nodules >1 cm in diameter regardless of the results of fine-needle aspiration biopsy.[7,8] The Philippine Interim Clinical Practice Guidelines for the Diagnosis and Management of Well-Differentiated Thyroid Cancer 2021 deviated from the ATA guidelines, primarily by recommending total thyroidectomy for all unifocal Bethesda category V and VI nodules exceeding 1 cm.[9]

In this systematic review, we examined the incidence rate, disease extent at diagnosis, recurrence rate, and DSM of DTC in Filipinos residing in the Philippines and other countries (Filipino immigrants) compared with other ethnic groups. The possible factors and mechanisms contributing to disease extent, recurrence and mortality of DTC in Filipinos were investigated.

METHODOLOGY

Search strategy and selection criteria

This review was performed according to the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.[10] This study was acknowledged by the Institutional Review Board. A systematic literature search until January 27, 2022, was performed in MEDLINE, Google Scholar, EBSCO, Cochrane, and Clinicaltrials.gov using combinations of the following keywords: "thyroid cancer" or "thyroid malignancy" or "thyroid carcinoma" and "*Filipino*" or "Philippines." We included cohorts and case control studies. No language restrictions were applied. Studies investigating PTC and FTC were included. Studies that included low-risk thyroid malignancy alone, anaplastic, medullary and other subtypes of thyroid malignancy were excluded. For studies involving Filipino immigrants residing outside of the Philippines, Filipino descent was defined as either birth in the Philippines or based on self-proclaimed primary race identification.

Data extraction and analysis

Two independent reviewers performed screening of titles and abstracts. Full-text articles of studies meeting all inclusion criteria were retrieved. Disagreements were resolved by consensus. Both reviewers assessed the risk of bias using the Newcastle-Ottawa Scale for cohort and case-control studies. Quality of the included studies were rated based on total score: poor quality for 0 to 2, fair quality for 3 to 5 and high quality for 6 to 9.

We extracted the following variables: ethnicity, incidence of PTC and FTC, presence of regional or distant disease at diagnosis, rate of disease recurrence or incomplete response and DSM. Incidence was reported per 100,000 person-years. The extent of disease at diagnosis or during primary surgery based on presence of lymph node metastases and distant metastases were obtained. Staging based on American Joint Committee on Cancer (AJCC) and ATA were not included due to changing definitions over the years.

StatsDirect Statistical Software 3.3.5 (StatsDirect Ltd) was used to determine the incidence rate ratio (IRR) between Filipino immigrants and non-Hispanic whites. Pooled proportions using the Stuart-Ord method was performed to compare the characteristics of thyroid malignancy in Filipinos, Filipino immigrants, and non-Hispanic whites. The fixed effects model (inverse variance) was utilized when heterogeneity based on I^2 is below 75%. When I^2 exceeds 75%, the random-effect model (DerSimonian-Laird) was used to achieve more conservative estimates of the 95% confidence intervals (CIs).

RESULTS AND DISCUSSION

Literature search yielded 1,852 studies. After removal of duplicates and screening of titles and abstracts, 26 articles were retrieved. Nine retrospective case controls and cohorts were deemed acceptable to be included in this systematic review. No randomized clinical trials were identified. The search flow diagram is presented in Figure 1.

Tables 1 and 2 summarize the characteristics of the included studies. The first group of studies was performed outside of the Philippines and contain data on the incidence and characteristics of thyroid malignancy in Filipinos

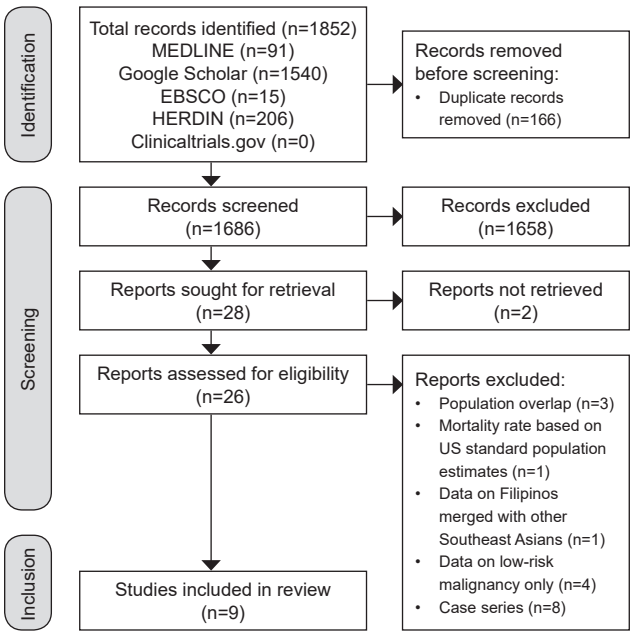

Figure 1. PRISMA flow diagram of search results.

who immigrated to other countries compared with non-immigrants or non-Hispanic whites (Table 1). The second group of studies was performed in the Philippines and present local data on the characteristics of DTC in Filipinos (Table 2).

The risk of bias of the included studies are summarized in Figure 2. All studies included were of fair to high quality. Only one study from the Philippines declared specific follow-up duration and presented DSM, which limits the analysis of disease outcomes.

Table 1. Characteristics of studies performed outside the Philippines

Study	Method	Source of data	Population
Clark 2006[7]	Retrospective, matched-pair analysis	Mount Sinai Hospital (Toronto) from 1983 to 2004	Filipino = 72 Matched control = 72
Kus 2010[8]	Retrospective cohort	Mount Sinai Hospital (Toronto) from January 1, 1984 to August 31, 2003	Filipino = 36 Non-Filipino = 463
Lee 2022[4]	Retrospective cohort	SEER* database from January 1, 1990 to December 31, 2014	Filipinos = 5,341 Non-Hispanic whites = 106,397 Non-Filipino Asians = 8,303
Megwalu 2021[38]	Retrospective cohort	California Cancer Registry from January 1, 2004 to December 31, 2015	Filipino = 2065 Non-Filipino Asian = 4327 Non-Asian = 30,181
Shah 2017[13]	Longitudinal cohort	Ontario healthcare system from January 1, 1997; observed until March 31, 2015	Southeast Asia = 203,361 East Asia = 364,288 Other Immigrants = 1,611,968 Nonimmigrants = 12,480,116
Spitz 1988[5]	Retrospective cohort	SEER* database from 1973 to 1981	Filipino = 193 Non-Hispanic white = 5,979 Puerto Rico Hispanics = 528 Blacks = 384 New Mexico Hispanics = 189 Chinese = 103 Japanese = 196

*SEER – Surveillance, Epidemiology, and End Results

Table 2. Characteristics of studies performed in the Philippines

Study	Method	Source of data	Population
Lo 2016[3]	Retrospective cohort	Philippine General Hospital from January 1990 to June 2014	Filipino = 728
Mendoza 2015[22]	Retrospective cohort	University of Santo Tomas Hospital from January 2007 to December 2011	Filipino = 225
Santiago 2021[21]	Retrospective cohort	Makati Medical Center from 2013 to 2017	Filipino = 115

	Selection				Comparability		Outcome			Total score	Quality
	Representative of exposed	Representative of non-exposed	Ascertainment of exposure	Outcome not present at start of study	Comparability of main factor	Comparability of additional factors	Assessment of outcome	Sufficient follow-up time	Adequacy of follow-up		
Clark 2006[7]*	★	★	★		★	★	★	★	★	8	High
Kus 2010[8]	★	★	★		★		★	★	★	7	High
Lee 2022[4]	★	★	★		★		★			5	Fair
Megwalu 2021[38]	★	★	★		★		★	★	★	7	High
Shah 2017[13]	★	★	★	★	★		★	★	★	8	High
Spitz 1988[5]	★	★	★		★		★			5	Fair
Lo 2016[3]	★		★				★	★		4	Fair
Mendoza 2015[22]	★	★	★		★		★		★	6	High
Santiago 2021[21]	★	★	★		★		★		★	6	High

*Modified NOS criteria for case-controls used

Figure 2. Newcastle-Ottawa Scale (NOS) risk of bias scoring of included studies.

Table 3. Incidence of differentiated thyroid cancer

Study	Filipino immigrants		Non-Hispanic whites	
	Male	Female	Male	Female
Papillary Thyroid Cancer				
Spitz 1988	5.2	12.5	1.5	3.9
Lee 2022	5.8	17.9	4.5	13.2
Pooled IRR	I^2 = 97.5% Random effects = 2.1 (95% CI 0.8 to 5.5), *p*=0.136	I^2 = 93.8% Random effects = 3.9 (95% CI 2.6 to 6.1), *p*<0.0001		
Follicular Thyroid Cancer				
Spitz 1988	1.0	4.0	0.4	0.9
Lee 2022	0.5	1.6	0.6	1.3
Pooled IRR	I^2 = 89.5% Random effects = 1.4 (95% CI 0.5 to 4.1), *p*=0.5581	I^2 = 0% Fixed effects = 3.8 (95% CI 3.4 to 4.3), *p*<0.0001		

Age-adjusted incidence rates reported per 100,000 person-years

Incidence of thyroid malignancy in Filipinos

In 2008, a nationwide survey estimated that 4.1% of Filipinos have nodular thyroid disease, although the etiology of these nodules were not identified.[11] We did not find a study on the incidence of DTC in the Philippines, but a report from the Philippine Cancer Society estimated that the age-adjusted incidence of thyroid malignancy was 3.4 per 100,000 person-years in males and 11.4 per 100,000 person-years in females from 2003 to 2007, with >90% of cases classified as DTC.[12]

We were able to find two retrospective studies from the United States (US) investigating the incidence of thyroid malignancy in Filipino immigrants compared with non-Hispanic whites (Table 3). Male Filipino immigrants had 2.1-fold (95% CI 0.8 to 5.5) higher incidence of PTC, although this was not significant (p=0.136). Among females, Filipino immigrants had significantly higher incidence of PTC with IRR of 3.9 (95% CI 2.6 to 6.1, p<0.0001) compared with non-Hispanic whites. Similar trends for incidence of FTC were noted, with Filipino immigrants having non-significant trend for higher incidence of FTC in males (IRR 1.4, 95% CI 0.5 to 4.1, p=0.5581) and significantly higher incidence of FTC in females (IRR 3.8, 95% CI 3.4 to 4.3, p<0.0001). A longitudinal cohort done in Ontario showed higher incidence of all histologic types of thyroid malignancy in Filipino immigrants with adjusted hazard ratio of 3.20 (95% CI 2.97 to 3.45) compared with non-immigrants.[13]

In a matched pair analysis of 72 Filipino immigrants and 72 non-Filipinos who presented with a thyroid nodule and underwent thyroidectomy, prevalence of DTC was 1.8-fold higher in Filipino immigrants (69.4%) compared with non-Filipinos (38.9%).[7] The incidence rate of DTC in Filipino immigrants in the US was almost 1.5-fold higher compared with the incidence of thyroid malignancy in Filipinos living in the Philippines. Filipino immigrants in the US also had a higher annual percentage increase in incidence of all subtypes of thyroid cancer (5.8%, 95% CI 2.9 to 8.7) from 2003 to 2010,[14] compared with an annual increase of 1.1% in males and 2.7% among females in the Philippines from 2003 to 2007.[12] These trends in incidence of thyroid malignancy may be due to under-reporting and low healthcare accessibility in the Philippines, in contrast to over-diagnosis of small, indolent tumors in developed countries.

Incidence of PTC and FTC were 2.4- to 3.1-fold higher in females compared with males across both ethnicities. Thyroid cancer is currently considered to be the only non-reproductive cancer with striking female predominance, with females having 3- to 4-fold higher incidence.[15] Although the exact molecular basis remains unknown, it is postulated that higher estrogen levels and higher risk for Hashimoto's thyroiditis may contribute to thyroid cancer pathogenesis in females.[16]

Using data from the California Cancer Registry from 1988 to 2004, Horn-Ross et al., found that the age-adjusted incidence rate of PTC was higher in Filipinas born in the US compared with Filipinas who were born in the Philippines then immigrated to the US (17.8 vs 13.3 per 100,000 person years, incidence rate ratio 0.7, 95% CI 0.6 to 0.9, p=0.02).[17] They postulated that the higher weight and body mass index in US-born Asian women may contribute to the increased risk for thyroid malignancy. The role of estrogen levels and parity on thyroid cancer risk remains to be determined.

Extent of disease at primary surgery

Characteristics of DTC in Filipinos were described in one study from the US, two studies from Canada, and three studies from the Philippines (Table 4). Pooled proportion of lymph node metastases during primary surgery was similar between the three groups: 0.3 (95% CI 0.2 to 0.4) in Filipinos, 0.3 (95% CI 0.3 to 0.3) in Filipino immigrants, and 0.3 (95% CI 0.3 to 0.3) in non-Hispanic whites. Distant metastases were reported in 0.1 (95% CI 0.03 to 0.2) of Filipinos, 0.03 (95% CI 0.02 to 0.03) of Filipino immigrants, and 0.02 (95% CI 0.01 to 0.02) of non-Hispanic whites.

AJCC and DeGroot staging systems were used in the studies. Presence of lymph node metastases upon initial surgery appears to be similar among Filipinos, Filipino immigrants and non-Hispanic whites. For PTC, which comprises 80% to 90% of all thyroid malignancies, 15% (range 5% to 34%) have extrathyroidal extension into adjacent soft tissues and 35% to 50% of excised neck lymph nodes have histologic evidence

Table 4. Extent of disease of well-differentiated thyroid malignancy in Filipinos, Filipino immigrants, and non-Hispanic whites

Study	Filipinos			Filipino immigrants			Non-Hispanic whites		
	Local	LN	Distant	Local	LN	Distant	Local	LN	Distant
Clark 2006				35/46	11/46	0/46	24/28	4/28	0/28
Kus 2010						0/33			
Megwalu 2021				1391/2065	614/2065	60/2065	21682/30181	8001/30181	498/30181
Pooled Proportions				0.7 (95% CI 0.6 to 0.7) I^2 = 33.4%	0.3 (95% CI 0.3 to 0.3) I^2 = 0%	0.03 (95% CI 0.02 to 0.03) I^2 = 25.8%	0.7 (95%CI 0.7 to 0.7) I^2 = 63.5%	0.3 (95% CI 0.3 to 0.3) I^2 = 52.9%	0.02 (95% CI 0.01 to 0.02) I^2 = 0%
Mendoza 2015	90/225	89/225	46/225						
Lo 2016	492/728	200/728	36/728						
Santiago 2021	88/115	18/115	9/115						
Pooled Proportions	0.6 (95% CI 0.4 to 0.8) I^2 =96.9%	0.3 (95% CI 0.2 to 0.4) I^2 =91.5%	0.1 (95% CI 0.03 to 0.2) I^2 =95.2%						

Table 5. Outcomes of well-differentiated thyroid carcinoma in Filipinos and Filipino immigrants

Study	Filipinos			Filipino immigrants			Non-Hispanic whites		
	Recurrence	Mortality	Follow-up	Recurrence	Mortality	Follow-up	Recurrence	Mortality	Follow-up
Clark 2006					0/46	32.3 ± 52.3 months			
Kus 2010				9/33 (0.3)		119.5 ± 53.4 months	44/463 (0.1)		
Megwalu 2021					101/2065	60 months		482/30181 (0.02)	60 months
Pooled Proportions					0.03 (95% CI 0.0005 to 0.09) I^2 = 75.7%				
Lo 2016	201/728 (27.6%)	4/728 (0.005)	62.4 ± 57.5 months						
Mendoza 2015	69/225 (30.7%)		At least 24 months						
Santiago 2021	47/115 (40.9%)		At least 24 months						
Pooled Proportions	0.5 (95% CI 0.02 to 0.9) I^2 = 99.7%								

of involvement.[2] Lymph node metastases at presentation of PTC do not seem to adversely affect survival, but it increases the risk of locoregional recurrence.[18] In contrast, FTC may have extensive local invasion but rare nodal metastases.[19]

Distant metastases were least frequent in non-Hispanic whites, followed by Filipino immigrants, and highest in Filipinos. In general, distant metastases are noted in 1% to 7% of patients at diagnosis of PTC[2] and 9.4% to 23% of FTC.[2,19] The higher rate of distant metastases in Filipinos compared with Filipino immigrants and non-Hispanic whites may be due to reporting bias towards more severe disease in the Philippines, since all data were reported by tertiary hospitals. Lack of access to healthcare services in the Philippines may have also contributed to these findings.

Recurrence after primary treatment

Recurrence was defined as the presence of biochemical or structural incomplete response and indeterminate response at least 2 years after thyroidectomy with or without radioiodine ablation. Disease recurrence was highest in Filipinos (pooled proportion 0.5, 95% CI 0.02 to 0.9), followed Filipino immigrants (0.3), and lowest in non-Hispanic whites (0.1) (Table 5).

In PTC, recurrent disease occurs in up to 30% of patients during the first ten years after treatment.[20] Management consists of thyroid surgery and radioactive iodine ablation (RAI). Extent of thyroidectomy and use of postoperative ablation varied among the studies included and may have affected the recurrence rate reported. For the studies done in the Philippines, RAI was done in 64%,[3] 80%,[21] and 100%[22] of patients. RAI significantly decreased recurrence in one study (OR 0.4, p<0.001)[23] but did not significantly affect recurrence in another study.[21] In the retrospective study by Kus, Filipino immigrants were found to have increased risk of thyroid cancer recurrence compared with non-Filipino patients (OR 6.9; 95% CI 2.3 to 21.1; p<0.001), even after controlling for sex, age, history of head and neck radiation therapy, family history of thyroid cancer, tumor size, tumor pathologic findings, stage of primary disease, use of radioactive iodine therapy, use of external beam radiation therapy and type of thyroid surgery.[8]

Disease-specific mortality

We found limited data on DSM. Mortality was highest in Filipino immigrants (pooled proportion 0.03, 95% CI 0.0005 to 0.09), followed by non-Hispanic whites (0.02), and lowest in Filipinos (0.005) (Table 5).

In general, patient survival in well-differentiated thyroid malignancy is excellent. Using data from the Multiple Cause of Death File of the National Center for Health Statistics of the US from 2003 to 2012, Nguyen et al., found that Filipino immigrants had significantly higher age-adjusted mortality rate due to thyroid cancer compared with non-Hispanic whites (1.7, 95% CI 1.5 to 1.9 vs 1.2, 95% CI 1.2 to 1.2 per 100,000 individuals, $p<0.0001$) and non-Filipino Asians (vs 1, 95% CI 0.9 to 1.1 per 100,000 individuals, $p<0.0001$). Furthermore, proportional mortality ratio compared with non-Hispanic whites was significantly higher for Filipino immigrants born in the Philippines than US-born Filipino immigrants (4.1, 95% CI 3.6 to 4.6 vs 2.6, 95% CI 1.6 to 4.1, $p<0.0001$).[24] The lower mortality rate from local Philippine data may be due to reporting bias, since Lo included patients with incomplete data,[3] such that patients lost to follow-up due to death from thyroid malignancy may not have been accounted for.

Environmental, genetic, and epigenetic mechanisms of disease

At present, we still do not know why Filipinos have higher incidence of DTC, higher disease recurrence and higher mortality rate compared with other ethnicities. Some studies suggest high iodine diet and possible radiation exposure in Filipino healthcare workers as possible mechanisms.[24] Environmental factors unique to the Philippines are yet to be investigated.

The genetic and molecular basis of thyroid malignancy have received much attention in recent years. Mutations in drivers of the MAPK signaling cascade, such as BRAF (MAP3K), RAS (small GTP-binding protein) and RET (receptor tyrosine kinase) have been identified as the main drivers of thyroid malignancy.[25] BRAF activating mutations account for 51% to 59.7% of PTCs and are strongly correlated with poor clinicopathological outcomes, such as extrathyroidal extension and lymph node metastasis, hence resulting in increased recurrence and mortality rates.[26] In Filipinos with conventional PTC, BRAF V600E mutations were found in 38.5%[27] and 70.6%.[28] Among Filipino immigrants in Hawaii with conventional PTC, 83.8% harbored the BRAF V600E mutation.[29] These rates are comparable with similar studies done in the US (50% to 88.2%),[30,31] Italy (37.5% to 62.2%)[32] and South Korea (79.7%).[33]

A group from California compared miRNA expression profiles of Filipino immigrants and European immigrants diagnosed with PTC. Compared with European immigrants, Filipino immigrants had significantly upregulated miR-4633-5p and significantly downregulated miR-491-5p and let-7. Higher miR-4633-5p have been associated with advanced thyroid cancer staging, while miR-491-5p and let-7 are tumor suppressors.[34] These findings suggest possible mechanisms for increased thyroid cancer incidence, distant metastases and recurrence in Filipinos.

Implications for management

The ATA has recommended a Risk of Recurrence Stratification System using tumor characteristics during initial surgery to guide aggressiveness of management of DTC. Patients are designated as low risk, intermediate risk, or high risk for disease recurrence based on the presence of extrathyroidal extension, vascular invasion, cervical lymph node metastases, distant metastases, incomplete tumor resection, tumor histopathology and postoperative thyroglobulin values.[6] In three studies from the Philippines, recurrence of PTC and DTC was significantly associated with multifocality, lymphovascular invasion, ATA high-risk stratification and detectable post-operative thyroglobulin and anti-thyroglobulin levels.[21–23] In Filipinos with DTC who underwent total or near-total thyroidectomy with post-operative RAI, incomplete response was noted in 8.3% of ATA low-risk patients, 53.7% of ATA intermediate-risk patients and 92.3% of ATA high-risk patients. Similar studies in the US and South America showed recurrence in 12% to 22% of ATA low-risk patients, 37% to 48% in ATA intermediate-risk patients and 69% to 84% in ATA high-risk patients.[35–37]

The higher incidence of DTC in Filipinos and Filipino immigrants suggest that thyroid nodules may need to be managed more aggressively in this ethnic group. Furthermore, the risk of disease recurrence appears to be higher than predicted by the ATA risk stratification system. Local data regarding DSM in Filipinos are still lacking. Additional prospective studies on the outcomes of thyroid malignancy in Filipinos are needed to provide definitive data on whether more aggressive treatment approaches are warranted in this ethnic group.

CONCLUSION

This systematic review supports the trend of increased incidence and recurrence of DTC among Filipinos and Filipino immigrants. This initial work can provide the basis for case registries that can give more comprehensive data on the incidence and prevalence of DTC in the Philippines. There is a need to evaluate the applicability of American Thyroid Association and European guidelines for thyroid cancer, in terms of risk assessment and appropriate management. In the setting of the newly released Philippine guidelines for thyroid carcinoma, prospective studies with active long-term follow-up is essential to detect any changes in the outcomes of DTC among Filipinos.

Limitations

Most studies included in this review are retrospective and are at high risk for reporting bias. Selection and attrition bias, especially in the studies conducted in the Philippines, may result in underreporting of extent of disease, recurrence and mortality. Studies included had heterogenous populations. Publication bias may have limited this review as well.

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

CRediT Author Statement
MDSJ: Conceptualization, Methodology, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization; **EPP:** Conceptualization, Methodology, Data Curation, Writing – review and editing, Supervision

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CASE REPORT

Swyer Syndrome Presenting as Dysgerminoma: A Case Report

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Abstract

Complete gonadal dysgenesis with 46,XY karyotype is a clinical condition characterized by the absence of testicular tissue but with the presence of typical Müllerian structures in a phenotypically female individual. The condition presents as primary amenorrhoea or delayed puberty. Eventually, malignant neoplasms may arise. We report a case of a 16-year-old Indian male with Swyer syndrome presenting with primary amenorrhoea and with an earlier diagnosis of a malignant dysgerminoma in the right ovary.

Key words: Swyer syndrome, dysgerminoma, amenorrhoea, gonadal dysgenesis

INTRODUCTION

Complete or pure gonadal dysgenesis (CGD) was first described by Dr. Gim Swyer in 1955, when he reported two women with tall stature, primary amenorrhoea, normal external genitalia, vagina and cervix, but with a karyotype of 46,XY.[1] The incidence of this eponymous condition is about one in 80,000. The process of testicular development, usually occurring in the second month of gestation, is regulated by several genes, the most crucial being SRY.[2] Inactivating mutations or deletion of SRY in the DNA-binding regions are present in approximately 15% of patients with Swyer syndrome.[3] Alterations in other genes, namely DAX1, WNT4, SOX9, SF1, and WT1, may also cause inhibition or mutation of SRY function,[4] resulting in streak gonads that do not secrete sex steroids or Anti-Müllerian Hormone (AMH). Hence, both the internal and external genitalia of such patients are phenotypically female. Patients with CGD and a 46,XY genotype are also at increased risk of developing gonadal tumours, namely, gonadoblastoma and dysgerminoma,[5] with an incidence of 20-30% and prophylactic gonadectomy is recommended.[6]

Dysgerminoma is the most common malignant germ cell tumour of the ovary. It can be found either in a pure form or mixed with other germinal elements.[5] The incidence of dysgerminoma is greater in younger women.[4] About 65% of dysgerminomas are at stage one at diagnosis; 85-90% of stage one tumours are unilateral, and 10-15% are bilateral.[5] Approximately five percent of dysgerminomas are found in phenotypic females with XY karyotype. Imaging of dysgerminoma shows multilobulated solid masses with well-defined fibrovascular septa and speckled calcifications.[7] On gross examination, they appear as firm, lobulated masses, while microscopically, they are composed of undifferentiated vesicular germ cells with clear cytoplasm and centrally placed regular nuclei. The morphology resembles that of a fried egg.[4]

We report a case of a 12-year-old phenotypical female presenting with dysgerminoma of the right ovary whose Swyer syndrome was recognized four years later.

CASE

A 12–year-old Indian female was referred to a local hospital due to a one month history of a gradually increasing, painless, right-sided abdominal swelling. There was no ulceration, fever, loss of body weight or impairment of function. On physical examination, her external genitalia were unambiguously female without any clitoromegaly and Tanner staging was prepubertal. Ultrasound of the abdomen revealed a large midline space-occupying lesion measuring 13 cm x 12 cm in the umbilical region, separate from the urinary bladder, having a heterogeneous echotexture and scattered echogenic specks (Figure 1). The contrast-enhanced computed tomography (CECT) scan of the abdomen was unremarkable. The laboratory results obtained are summarized in Table 1.

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Table 1. Baseline blood parameters

Laboratory parameters	Obtained value	Reference value/range
Haemoglobin (g/L)	105	116-150
Urea (mmol/L)	6.4	2.1-8.5
Creatinine (µmol/L)	57.47	53-97.2
Total Serum Bilirubin (µmol/L)	9.75	1.71-20.5
Serum Alkaline Phosphatase (U/L)	191	44-147
Alpha fetoprotein (µg/L)	1.41	10-20
Beta-hcG (IU/L)	4.30	<5
Carcinoembryonic antigen (µg/L)	1.90	0-2.5
CA-125 (kU/L)	67.2	<46
Lactate dehydrogenase (µkat/L)	10.27	2.33-4.67

Laparotomy was performed due to suspicion of malignancy. Intraoperative findings showed evidence of a tumour (15 cm x 12 cm x 10 cm) weighing 500 grams, arising from the right ovary and twisted from the right cornu. The left ovary was found to be very small for age. A gross histopathological examination of the resected specimen confirmed a right ovarian tumour, 14 cm in diameter. The tumour was solid with greyish-brown haemorrhagic, with infarcted areas (probably due to torsion), but with an intact capsule. On microscopy, the more significant part of the tumour was infarcted and showed ghost outlines of sheets, nests, cords and trabeculae of round or oval tumour cells. At the

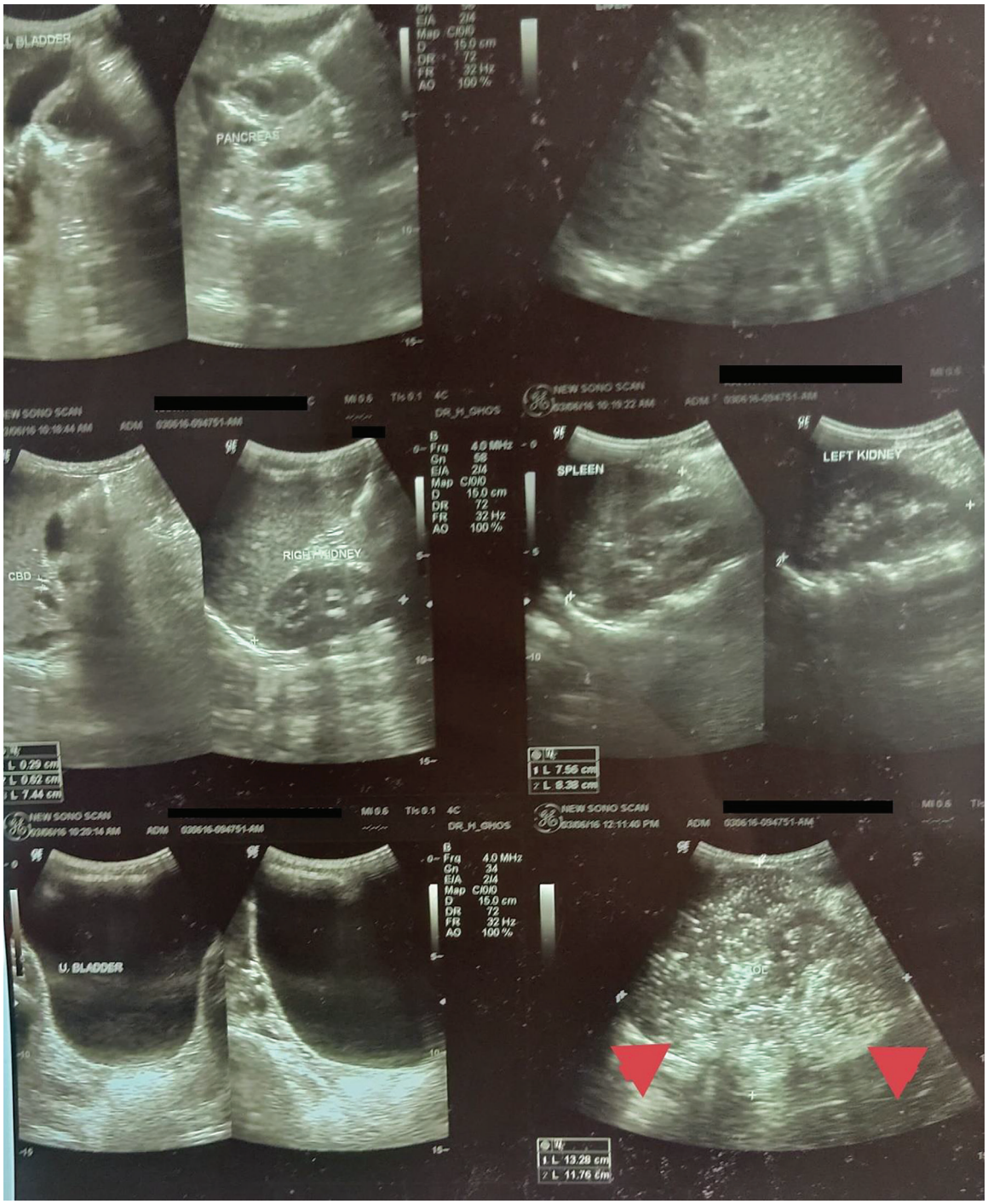

Figure 1. Ultrasound scan of abdomen of the 12-year-old patient. The red arrowheads indicate an area of increased echogenicity suggestive of a space-occupying lesion. The other organs appear normal.

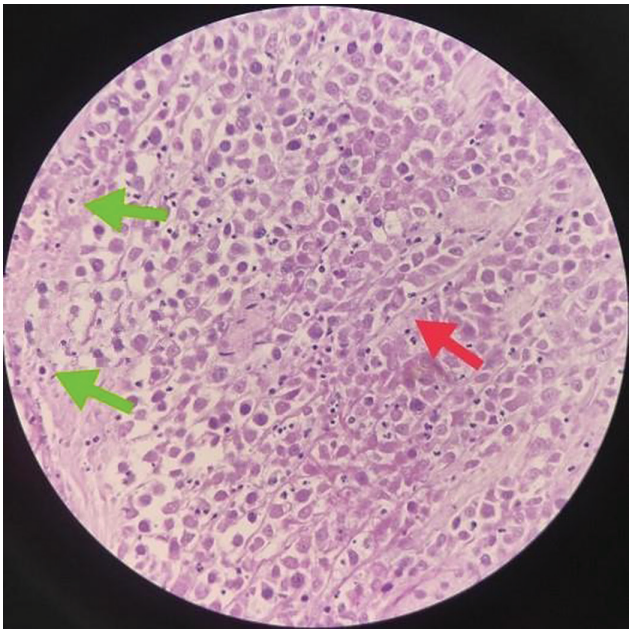

Figure 2. H&E stain of the resected specimen of the right ovary *(40x)*. The periphery is occupied by viable cells with distinct features *(green arrows)*. The central infarcted part of the specimen demonstrates ghost outlines of cells arranged in sheets and cords.

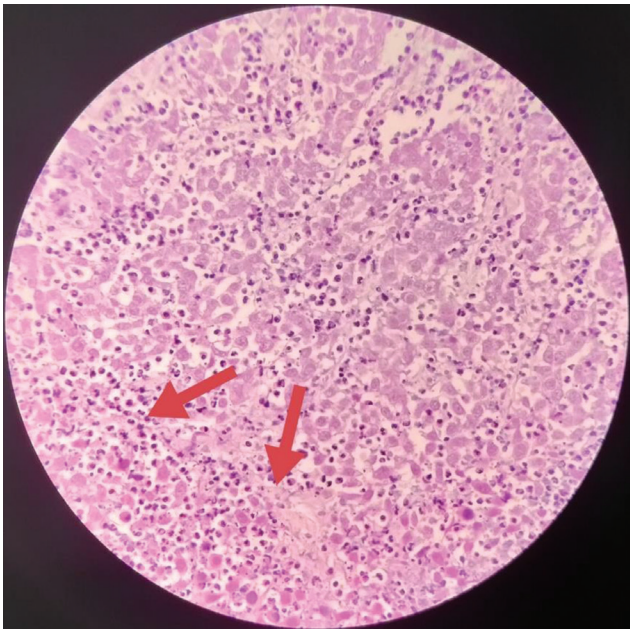

Figure 3. H&E stain of the resected specimen of the right ovary *(100x)*. The pale pink fibrous stroma is densely infiltrated with hyperchromatic inflammatory cells *(red arrows)*.

periphery, the viable tumour cells had round to oval vesicular nuclei, prominent nucleoli and amphophilic cytoplasm (Figure 2). The nests of the tumour were surrounded by a delicate fibrous stroma infiltrated by inflammatory cells (Figure 3). Extensive areas of haemorrhage and foci of calcification were seen. Lymphovascular and capsular invasions were found; however, metastatic deposits were absent. Immunohistochemistry was positive for Oct4 and CD117 focally and negative for CK and CD30. As a part of her treatment protocol, she received four cycles of intravenous bleomycin, etoposide and cisplatin (BEP) chemotherapy. The recovery was uneventful until the age of 16, when she was referred to our endocrinology department for evaluation of delayed puberty. On examination, her height was 1.61 m (75th percentile) and her arm span was 1.7 m. The findings of Tanner staging and examination of external genitalia were identical to previous records. The results of biochemical investigations are summarized in Table 2.

The whole abdominal ultrasound scan (USG) showed a left ovary (2 cm x 0.7 cm) with few tiny follicles, a uterine volume of 7.8 cc, and a non-visualized right ovary.

Table 2. Laboratory parameters after chemotherapy

Laboratory parameters	Obtained value	Reference value/range
Follicle-stimulating hormone (FSH) (IU/L)	89.4	2.5-10.4 (follicular)
Luteinising Hormone (LH) (IU/L)	56.7	1.9-12.5 (follicular)
Prolactin (µg/L)	6.3	1.9-25
Estradiol (pmol/L)	40.09	73-367 (follicular)
Inhibin B (pg/ml)	7.17	15-300
AMH (pg/ml)	2.18	0.9-9.5

Magnetic resonance imaging (MRI) scan of the abdomen and pelvis revealed a small, hypoplastic uterus (Figure 4). The right adnexal region showed no more lesions, and the left ovary was almost normal. However, cytogenetic studies revealed a 46,XY karyotype. No other gene sequencing studies were performed due to funding limitations. When the result was conveyed, the patient and her mother expressed wishes for a hysterectomy and total abdominal hysterectomy with left-sided salpingo-oophorectomy was performed. Histopathological examination of the resected left-sided adnexa revealed a streak gonad (Figure 5) with no evidence of malignancy.

The patient was initiated on 2 mg once daily oral estradiol valerate, to be continued till the age of menopause, along with calcium and vitamin D supplementation to help improve bone mineral density. Despite having a safer side-effect profile, transdermal estradiol could not be offered due to cost and availability constraints. There was no further indication for progesterone after the surgery. A bone mineral density testing was scheduled only after attainment of Tanner Stage 5 of pubertal development.

The mother and the patient were counseled about the condition's future social and psychological implications. The child identified more as a female; hence, her mother was advised to support the perspective through mental and emotional support. It was explained that regular sexual intercourse was possible; however, our patient would not be able to conceive. Potential options like pregnancy by donor egg could be explored. However, the success rate might be less owing to the inherent lack of estrogen and the presence of an infantile uterus.

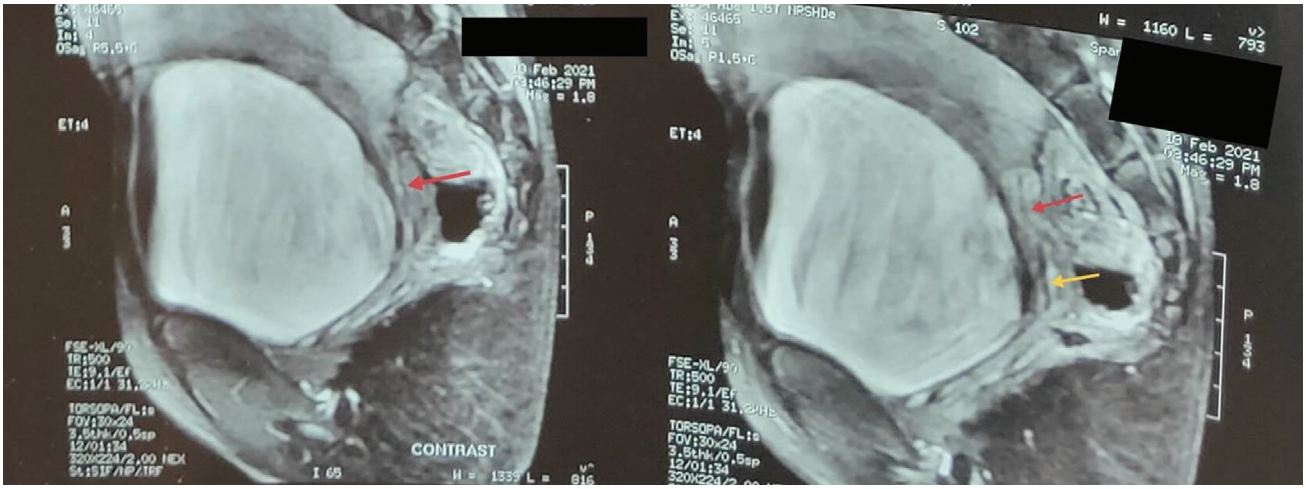

Figure 4. Sagittal cuts of MRI scan of the pelvis of the patient at the age of 16 years. The body of the uterus posterior to the bladder is hypoplastic with a slit-like uterine cavity *(red arrow)*. The length of the vagina is normal *(yellow arrow)*.

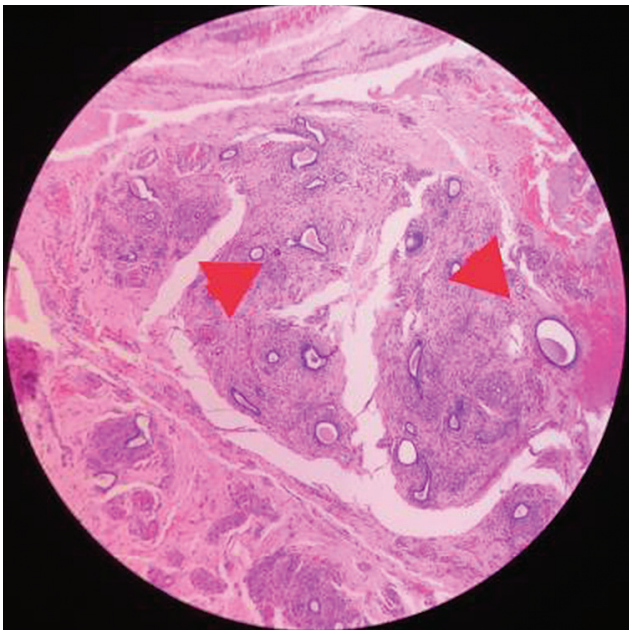

Figure 5. H&E stain of the left ovary *(100x)* obtained after total abdominal hysterectomy with left-sided salpingo-oophorectomy. Dense inflammatory infiltrates and atretic ovarian follicles containing colloid-like secretions dominate the field *(red arrowheads)*. Normal ovarian structures are replaced by a pale, fibrous stroma and narrow trabeculae. Dysplastic and anaplastic changes suggestive of malignancy are absent.

Follow-up was done six months after initiation of estradiol therapy. There were no significant complaints. On clinical examination, there has been a progression of pubertal features from Tanner stage B1 to B2.

DISCUSSION

Complete gonadal dysgenesis, a condition also called Swyer syndrome, is associated with a complete lack of androgenisation of the external genitalia and persistence of Müllerian structures,[3] owing to the lack of testosterone and AMH, the two testicular hormones involved in fetal sex differentiation. The gonads are usually hypoplastic without germ cells. The diagnosis is usually not suspected until puberty, when the patients complain of the absence of thelarche and menarche.

Our patient, a 12-year-old with phenotypically female external genitalia, presented with an enlarging abdomen, similar to the presentations observed by Caponetti et al.,[8] and Jonson et al.[9] The median age of diagnosis of Swyer syndrome is usually around 17 years.[10] In this patient, there were no features of virilisation (clitoromegaly, deepening of voice), as in the cases reported by Alam et al.,[11] Moreira et al.,[12] and Trovilion et al.[13] No other symptoms or signs such as confusion, pain, fever, were present in our patient, which contradicts the findings of Russo et al.,[14] where a 14-year old girl presented with lumbar pain and polyuria. Although rare, familial cases of Swyer syndrome have been reported previously.[15] Our patient, however, had no family history of consanguinity or other co-morbid conditions, as found in some other reports,[16,17] The patient's Tanner stage of was prepubertal which is typical of this syndrome.[6,18] However, spontaneous breast development and menstruation have also been reported as exceptions.[6,19,20]

Preoperatively, CA-125 was increased in our patient while the other tumour markers were normal, namely, b-hcG,[21] LDH[5] and AFP.[22] Although the excised ovarian tumour was unilateral and did not show any evidence of local spread or metastases, several cases of disseminated malignancy have been reported previously.[23,24]

Histopathological examination of the resected specimen revealed a dysgerminoma restricted to the right ovary, positive for Oct4 and CD117. In contrast to the finding reported by Anwar et al.,[25] it had not invaded the fallopian tube and did not show any other germ cell elements.[8] Despite placental alkaline phosphatase being a relatively specific marker for dysgerminoma as documented in several reports,[1] only serum total alkaline phosphatase

was performed in our patient, which was above the reference range.

Hypergonadotropic hypogonadism and hypoplastic female internal genitalia without testicular remnants have been unanimously reported in almost all cases of Swyer syndrome. Some reports have, quite peculiarly, demonstrated testicular remnants[26] and genotypic mosaicism in some patients suffering from the condition.[21] Our patient's clinical and biochemical status post-treatment has been unremarkable, and she is continuing her hormonal therapy. There have been some instances where there have been adverse outcomes in the form of persistently elevated tumour markers,[13] non-response to therapy,[27] emergence of a new pathology[26] and death.[15]

Differentiation of gonads occurs after the sixth week of gestation. They form testicular tissue under the influence of SRY, SF1, WT1 and SOX9 genes. However, in our patient the effects of the Y chromosome were probably overshadowed by genetic mutations, resulting in dysgenesis of the gonads in the undifferentiated stage to form ovarian structures. Masculine characteristics failed to develop, leading to the child being raised as female and the initial pelvic mass being suspected to be primarily an ovarian tumor.

The differential diagnoses of Swyer syndrome include complete androgen insensitivity syndrome and Mayer-Rokitansky-Kusterhauser syndrome. Complete androgen insensitivity was excluded as our patient had AMH levels towards the lower limit of normal, contrary to being well within the reference range.[4] Patients with androgen insensitivity syndrome have a female phenotype and normal breast development but with morphologically normal testes (which may be undescended) and no Müllerian structures. The absence of the uterus is also recently being considered as a criterion for diagnosing this condition.[28] Mayer-Rokitansky-Kusterhauser syndrome, another cause of primary amenorrhoea, affects 1 in every 5000 live females. The significant features of this syndrome are varying degrees of Müllerian duct and vaginal aplasia, along with a rudimentary uterus. However, their sexual characteristics and genotype are similar to that of a female.

Our patient manifested Swyer syndrome initially at the age of 12 years, which was not recognized till four years later. Thus, it emerged as a case of delayed diagnosis at late puberty, similar to previous reports where an opportunity for intervening at an earlier stage was overlooked. Hence, this adds to the existing cases warranting improvement in management strategies for the syndrome. It highlights the need to consider gonadal dysgenesis as a differential diagnosis in adolescent phenotypical females without thelarche, menarche, and adnexal masses.

CONCLUSION

Patients with complaints of delayed puberty and investigations revealing ovarian germ cell tumours should be promptly evaluated to rule out gonadal dysgenesis. Karyotyping should be mandatory even in other apparent causes of hypergonadotropic hypogonadism like chemotherapy.

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

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

CRediT Author Statement
SST: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration; **SC:** Methodology, Software, Investigation, Resources, Data Curation, Writing – original draft preparation, Visualization, Project administration; **ND:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration; **DH:** Conceptualization, Validation, Formal analysis, Investigation, Writing – review and editing, Supervision; **AB:** Conceptualization, Validation, Formal analysis, Writing – review and editing, Supervision; **PC:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration; **NS:** Conceptualization, Validation, Formal analysis, Writing – review and editing, Supervision,; **SG:** Conceptualization, Validation, Writing – review and editing, Supervision.

Author Disclosure
The authors declared no conflict of interest.

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CASE REPORT

Turner Syndrome and Neurofibromatosis 1: Rare Co-Existence with Important Clinical Implications

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Abstract

A 16.5-year-old Indian female presented with secondary amenorrhoea, cubitus valgus, scoliosis and multiple lentigines on the face. Karyotyping revealed mosaic Turner syndrome (TS) with 45, X/46, X iXq. She also had multiple café-au-lait macules and axillary freckles but no neurofibroma and did not fulfil the classic criteria for diagnosis of Neurofibromatosis-1 (NF1). Many of her macules were smaller than 15 mm in diameter, which might be due to her hypoestrogenic state. However, exome-sequencing found a pathologic variant consistent with NF1. She was started on daily oral estrogen, and oral progesterone for 10 days every month with close monitoring for neurofibroma and/or glioma expansion. Co-occurrence of NF1 and TS is extremely rare, TS and NF1 can both affect growth and puberty, cause different cutaneous and skeletal deformities, hypertension, vasculopathy and learning disabilities. Our case highlights the need for genetic testing in some cases with NF1 who do not strictly fulfil the NIH diagnostic criteria. We also emphasize the need for close monitoring during therapy with growth hormone, estrogen and progesterone due to the potential risk of tumour expansion in NF1.

Key words: Turner Syndrome, Neurofibromatosis-1, NF1, Neurofibromatosis-Noonan syndrome

INTRODUCTION

Turner syndrome (TS) and neurofibromatosis 1 (NF1) are two distinct genetic disorders and it is extremely rare for an individual to have both. Nevertheless, the presence of both genetic disorders in a single individual has important clinical implications. Although the genetic mechanisms causing TS and NF1 are not related, both disorders affect growth and puberty, and have cutaneous, skeletal and cardiovascular manifestations. To the best of our knowledge, only five cases of coexistent TS and NF1 are reported in literature as of this writing and almost all of them presented with classic clinical features of both NF1 and TS.[1-4]

In this report, we describe the case of a girl who did not have the classic presenting features of TS or NF1, but genetic tests revealed both these disorders and therefore required close supervision while receiving hormonal replacement. The case highlights important clinical considerations in the diagnosis and management of this dual pathology.

CASE

A 16.5-year-old Indian female presented with secondary amenorrhea for six months. She had spontaneous thelarche at 9 years of age and menarche at 11 years of age, following which she had regular menstrual cycles for five years. She had a history of pulmonary tuberculosis 2 years prior, for which she received antitubercular pharmacotherapy for six months, after which she was declared cured. She was born at term from a non-consanguineous marriage and her perinatal history and her childhood development were unremarkable. At the time of presentation, she was a student of the tenth standard with average scholastic performance. She was lean, had no clinical evidence of hyperandrogenism and had received no treatment before her consultation. There was no history of recent weight loss, chronic stress or malnutrition and she had no history of galactorrhea, headache, seizures or visual deficit.

On examination, her height was 147 cm (between 3rd to 10th percentile; Height SDS: -1.59 SD, Indian Academy of Pediatrics 2015 growth chart references, Upper segment: Lower segment ratio = 0.9:1), her target height being 165 cm; her body weight was 55 kg (between 75th to 97th percentile, Weight SDS = + 0.51), BMI 23.8 kg/m^2 (between 75th to 97th percentile, BMI SDS: +1.06).[5] She had sinus tachycardia with a heart rate of 120/min and had stage 1 hypertension with clinic BP of 136/88 mm Hg. There was mild scoliosis with convexity to the right. She had a grade 1b goitre and grade 2 acanthosis nigricans. She had

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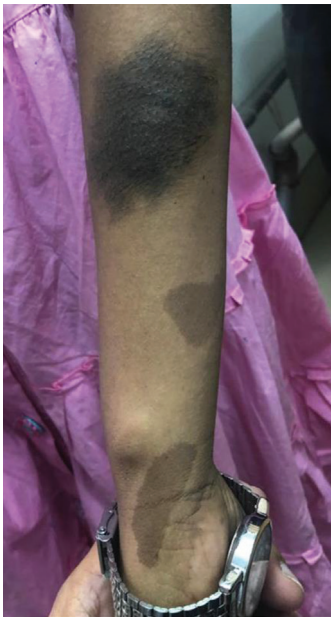

Figure 1. Multiple café-au-lait spots on the forearm.

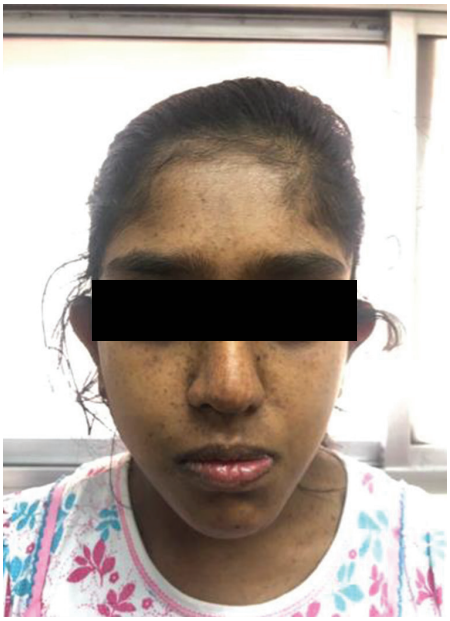

Figure 2. The face of the patient showing multiple lentigines and low set ears.

Figure 3. Cubitus valgus.

multiple café-au-lait macules with dimensions ranging from 5 to 25 mm distributed over her arms, thighs and backs (Figure 1), multiple lentigines over her face (Figure 2) and axillary freckling. She had cubitus valgus (Figure 3) and short fourth metacarpals. Systemic examination including cardiovascular, neurological, respiratory and abdominal examination revealed no abnormalities. Tanner's sexual maturity rating for her was B5P2A0 and she had unambiguous female genitalia with no clitoromegaly or hirsutism.

Initial investigations aimed at evaluating the etiology for short stature revealed normal results for most routinely tested parameters (Table 1). X-ray of her left wrist and hand showed a bone age of 17 years, which corroborated her chronologic age (Greulich and Pyle's atlas). She had subclinical hypothyroidism with TSH 5.6 mIU/ml, normal free T4, and TPO antibody was positive. She had evidence of primary ovarian failure with low estradiol inspite of high FSH and LH.

The clinical findings of proportionate short stature, secondary amenorrhea, cubitus valgus, multiple lentigines over face, scoliosis, hypertension and high FSH levels prompted further evaluation for Turner Syndrome. Karyotype of peripheral blood cells revealed mosaic TS with isochromosome Xq - 45, X [27]/46,X,i(X)(q10)[03] (Figure 4). Following a diagnosis of TS, relevant investigations to screen for comorbidities and complications known to be common in TS were done (Table 1).

The index case had a total of six café-au-lait macules out of which only four had diameters exceeding 15 mm. She also had evidence of axillary freckling. There were no subcutaneous or plexiform neurofibromata and upon slit lamp examination, she had no Lisch's nodules or distinctive osseous lesions like sphenoid dysplasia or tibial pseudoarthrosis. There was no known history of neurofibromatosis in any of her first-degree family members. Thus, she did not fulfil the classic criteria for a diagnosis of NF1, which was chiefly due to the smaller size of her café-au-lait macules than the cut-off of 15 mm required for diagnosis in the post-pubertal age.[6] However, due to strong clinical suspicion, clinical exome sequencing was done which revealed a heterozygous single base pair deletion in exon 21 of the NF1 gene (chr17: g.29556477delA), which was a pathogenic variant consistent with NF1. Home blood pressure monitoring confirmed persistent stage 1 hypertension. Screening for phaeochromocytoma through 24-hour urinary fractionated metanephrines (metanephrines and normetanephrines) was done with normal results. MRI of the brain revealed no optic nerve glioma or CNS tumours.

She was started on estradiol valerate 2 mg daily and medroxyprogesterone 10 mg daily for 10 days every month which induced regular menstrual cycles. She was counselled regarding the poor prospect of future fertility and offered the option for oocyte cryopreservation. During therapy with estrogen and progesterone, she was closely monitored for any new appearance of neurofibromata or worsening of visual acuity or headache for the possibility of optic glioma expansion though she did not develop any of these in her two years of follow-up. She was advised against daily estrogen-progesterone combined pills as a therapeutic strategy to reduce progesterone exposure since there is evidence suggesting the permissive role of progesterone on neurofibroma expansion.

Her bone age was 17 years and X-ray of her knees revealed fusion of her upper tibial and distal femoral epiphyses and therefore she was not initiated on recombinant growth

Table 1. Laboratory investigations and radiology findings

Parameter	Values	Reference range
Hb (g/dl)	12.5	12 – 15.5
WBC count (10^3/ul)	7.3 Neutrophil 52.3 % Lymphocyte 35.6% Eosinophil 3.4% Monocyte 8.1% Basophil 0.6%	4,500 – 15,000
Platelet count (10^3/ul)	287	150 - 450
Urea (mg/dl)	32	17 - 43
Creatinine (mg/dl)	0.66	0.2 – 1.4
FBS (mg/dl)	79	70 – 100
PPBS (mg/dl) 2 hr post 75 g glucose	133	70 - 140
HbA1c%	5.1	
Lipid profile	Total Cholesterol 167 mg/dl LDL cholesterol 104 mg/dl HDL cholesterol 48 mg/dl Triglycerides 138 mg/dl	
LFT	Bilirubin 0.43 mg/dl ALT 31.1 U/L AST 28.8 U/L ALP 135 U/L GGT 22 U/L Albumin 4.04 g/dl Globulin 3.47 g/dl	0.3 – 1.2 3 – 35 3 – 35 33 – 98 5 – 38 3.5 – 5.2 3 – 4.2
Urine R/E	pH: 5 Pus cells: 5 – 6 /hpf No casts/ RBC/ protein/ bacteria	
Serum bicarbonate (mEq/L)	25	22 – 29
Serum Calcium (mg/dl)	9	8.8 – 10.5
Serum Phosphorus (mg/dl)	4	2.5 – 4.5
25(OH)D (ng/ml)	27.8	> 20
USG abdomen and pelvis	Uterus: vol 24.8 cc, endometrial Thickness 3 mm Ovaries: Polycystic in appearance; Left ovary 8.2 cc, Right ovary 8.4 cc Kidneys and urinary tract: Normal	
FSH (mIU/ml)	89.2	1.5 – 11.2
LH (mIU/ml)	22.3	2 – 10
Estradiol (pg/ml)	9.5	27 – 254
Thyroid function tests	TSH (mIU/ml): 5.6 Total T4 (ug/dl): 7.6 Total T3 (ng/dl): 0.99 Anti-Thyroid peroxidase Antibody (U/L): 545	0.3 – 4.5 4.5 – 12 60 – 200 < 34
IGF-1 (ng/ml)	342	226 - 903
Anti-tissue trans-glutaminase IgA Ab	1.3 U/ml Total IgA: 2.7 g/l	< 4 U/ml 0.8 – 3.7
Bone Age (Left hand X ray)	17 yrs	
Karyotype (30 cell)	45,X [27]/46,X,i(X)(q10)[03]	
ECG all leads	Normal sinus rhythm, HR 120 / min	
Echocardiography	Normal cardiac chambers and valves, LVEF 67%	
Cardiac MRI	Aortic size index 1.7 cm/m^2 No abnormality detected in cardiac chambers, valves, ascending or descending aorta	
Pure tone Audiometry	Normal hearing thresholds in both ears	
DXA (Lunar Prodigy) scan for BMD	Z score at AP spine (L1 – L4): -2.3 Left femur neck: - 2.1. Left forearm: -2.4	
IQ test (Weschler's adult intelligence scale)	Verbal IQ scaled score: 95 Performance IQ scaled score: 78	

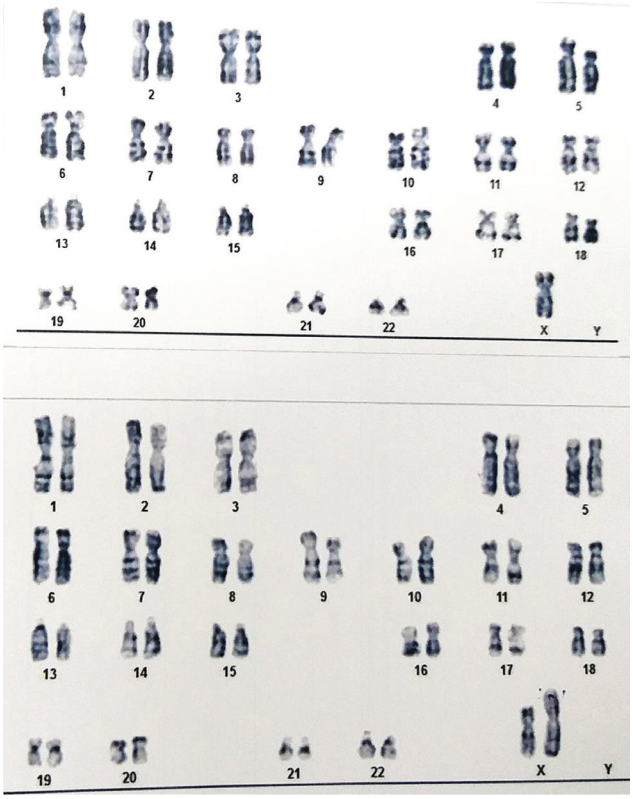

Figure 4. Karyotype 45,X[27]/46, X,i(X)(q10)[03].

hormone therapy. She was given calcium 500 mg/day and cholecalciferol 2000 IU/day for bone health. She was started on metoprolol 25 mg once daily for her hypertension and sinus tachycardia to achieve a target heart rate of 60/min and SBP less than 130 mm Hg. She was periodically monitored with echocardiography and cardiac MRI for aortic root diameter and aortic size index, annual testing of liver function and metabolic profile, pure tone audiometry and BMD-DXA.

Written informed consent was obtained from the patient and her parents for publication of this case report and images of the patient and her genetic tests.

DISCUSSION

The coexistence of NF1 and TS is extremely rare. Due to phenotypic similarities between the two syndromes like café-au-lait macules, many of the published cases presented as diagnostic dilemmas.[2-4] However, in all these cases, the diagnosis of NF1 could be made using the NIH diagnostic criteria.

Our case presented with secondary amenorrhea, short stature, cubitus valgus, scoliosis and short fourth metacarpals, along with multiple lentigines over the face. Although she had some café-au-lait macules and evidence of axillary freckling she did not fulfil the classic criteria required for a diagnosis of NF1, many of her café-au-lait macules were small and did not meet the size criteria of 15 mm required for a diagnosis of NF1 post-pubertally.

It is possible that the hypoestrogenic state due to premature ovarian failure within a few years of attaining puberty inhibited the growth in the size of her macules. Though NF1 is mostly a clinical diagnosis, genetic testing was done for her since there was a high clinical suspicion for NF1, which confirmed a pathogenic variant for NF1

Turner syndrome is one of the most common aneuploidies, seen in one in every 2,500 live births.[7,8] Neurofibromatosis is an autosomal dominant neurocutaneous disorder and the more common variety is NF1 with a prevalence of 1 in 3,000-4,000.[9,10] The diagnosis of TS is based on peripheral blood karyotype showing numerical or structural aberrations of one of the two X chromosomes which can be classic TS (45,X); mosaicism of 45,X with other cell lines and structural abnormalities of X chromosome.[8,11] NF1 is diagnosed based on a set of criteria established by the National Institutes of Health (NIH) which include the presence of multiple café-au-lait spots, Lisch nodules on the iris, optic glioma, axillary freckling, dermal neurofibromas, or distinctive skeletal abnormalities like sphenoid wing dysplasia and/or family history of a first-degree relative with NF1. Two or more must be present in specified numbers to establish the diagnosis.[6] NF1 is caused by pathogenic loss-of-function mutations in the tumour suppressor NF1 gene found on chromosome 17q11.2.[9] Though etiologically unrelated, the presence of the two diseases together can have important clinical implications.

Even though our index case had short height with respect to her target height, she had a height between the 3rd to 10th percentile for healthy Indian girls, and a normal height velocity for age. This, along with the normal progression of breast development and spontaneous menarche in her did not cause much concern to her or her caregivers leading to an overall delayed presentation. Short stature can be a manifestation of both TS and NF 1 but is rarely seen in NF1 alone.[6,7] Short stature in TS is due to several factors including SHOX haploinsufficiency, hypoestrogenism and concomitant disorders like hypothyroidism and celiac disease. Short stature in NF1 may be seen due to growth hormone deficiency or rarely, deficiency of multiple pituitary hormones due to compressive effects of a CNS tumour or following surgery or radiotherapy.[12]

This girl's karyotype revealed the presence of mosaicism of 45, X with 46,X,iX, which explains the spontaneous puberty and the lack of typical Turner phenotype like webbing of the neck or lymphedema.[13] Our case had sparse pubic and axillary hair. Though adrenarche is expected to be normal in TS, however, some studies suggest normal adrenarche but delayed pubarche in TS due to lack of ovarian conversion of DHEAS to active androgen following primary ovarian failure in TS.[14] NF1 is a known risk factor for isosexual precocious puberty in up to 3% of cases with NF1 which is sometimes, but not always, related to the presence of optic nerve gliomas, neurofibromas or other CNS tumours that impinge on neural pathways that inhibit hypothalamic GnRH pulse generator in childhood.[15] Our patient did not have any optic glioma or CNS tumours close to the hypothalamus and had an age-appropriate appearance of pubertal features till she developed premature ovarian insufficiency.

Due to delayed presentation after epiphyseal fusion of long bones, the index case did not receive rhGH therapy. Growth hormone therapy in TS has been postulated to increase the size of melanocytic nevi, though transformation to melanoma is not reported. GHR has been seen to be expressed in plexiform neurofibromas, which are known to be precursors of malignant peripheral nerve sheath tumours.[16,17] The use of rhGH in cases with NF1 is theoretically fraught with the risk of exacerbating the probability for nerve sheath and CNS tumours. However, available data do not support an increased risk of intracranial tumours among NF1 patients receiving GH therapy.[18] Both TS and NF1 are associated with scoliosis, the degree of which might be exacerbated with rhGH. Patients with NF1 and TS receiving rhGH must be closely observed for potential risk of neurofibroma enlargement and worsening of scoliosis.

The effects of estrogen and progesterone treatment on the neurofibromas is another area of concern. It is postulated that subcutaneous and plexiform neurofibromas increase in size and have an increased potential for malignant transformation during puberty and pregnancy, though this has been refuted by some studies.[19-21] Also, females with NF1 possibly have a greater propensity to develop vision loss due to optic glioma than males, as was seen in some reports.[22] This has been attributed to estrogen-mediated activation of microglia and a gender-specific role for cAMP regulation in gliomagenesis.[23-25] Girls with TS are expected to receive lifelong estrogen and progesterone supplements which may lead to a possible increase in the risk for neurofibroma expansion or malignant transformation. Studies have confirmed the presence of progesterone receptors in the majority of neurofibromas and increased proliferation rates of Schwann cells under the influence of progesterone.[26] However, estrogen receptors have been found in very few neurofibromas.[26] Gonadal hormones may lead to neurofibroma development, acting via a noncanonical pathway through GPER-1.[27] Case reports also demonstrate increased tumour growth in girls receiving depot progesterone preparations, but not in those receiving combined oral contraceptive pills.[27,28] Since the effects of progesterone are more established in girls and women with TS and NF1, use of progesterone should preferably be restricted to a maximum of ten days every month rather than a daily combined estrogen plus progesterone pill. Close monitoring is warranted in these women for any increase in the number and size of neurofibroma, any new appearance or worsening of neurologic symptoms and worsening of visual acuity due to progression of optic glioma.

Though hypertension can be seen in TS secondary to coarctation of the aorta, renal failure or as part of metabolic syndrome, the onset of essential hypertension

at a young age is also common.[7,8] Sinus tachycardia due to dysautonomia is also seen in TS, which increases the risk for aortic dissection. On the other hand, hypertension in NF1 needs screening for the presence of pheochromocytoma. Our patient had hypertension with sinus tachycardia. Secondary etiologies were ruled out and she was started on beta-blockers to control her blood pressure and heart rate. TS is known to also increase chances of aortic dissection and is also associated with aortic valve disorders and coarctation of the aorta. Mutations in neurofibromin can also lead to abnormal endothelial and vascular smooth muscle development.[28] The most common vasculopathy in NF1 is renal artery stenosis, followed by coarctation of the abdominal aorta.[29,30] Although the frequency of vascular anomalies in NF1 is low, the concurrent presence of TS and NF1 is expected to significantly enhance the risk for aortic vasculopathy.

Other clinical features common to TS and NF1 are learning disabilities and osseous anomalies in NF1 like bone cysts and dysplasia, which could contribute to craniofacial deformities and hearing defects. Bone cysts or dysplasia can also interfere with the interpretation of bone density by DXA scan which is recommended for osteoporosis screening for all girls with TS. The clinical presentation of NF1 with TS may mimic Neurofibromatosis-Noonan syndrome and Noonan-syndrome-with-multiple-lentigines, previously known as LEOPARD syndrome, due to phenotypic similarities between TS and Noonan syndrome.[31,32] However, karyotype analysis and genetic testing confirmed our index case to have NF1 coexisting with TS.

This was an extremely rare case of the concurrent presence of two distinct genetic disorders -TS and NF1, both of which affect growth, puberty and multiple organ systems. In our case, the café-au-lait macules and neurofibroma did not grow significantly to a considerable size, likely due to the hypoestrogenic state and thus did not classically meet the diagnostic criteria for NF1, which was eventually confirmed through exome sequencing. Genetic testing is indicated for NF1 diagnosis in patients with high clinical suspicion but not fulfilling the NIH criteria. For patients with both NF1 and TS receiving rhGH therapy and gonadal hormones, periodic screening of comorbidities and close monitoring for an increase in the size of macules and growth of neurofibroma and optic glioma is indicated. The use of progesterone should be restricted to a fixed number of days every month rather than daily therapy to minimise the risk of tumour expansion.

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

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

CRediT Author Statement
SM: Conceptualization, Methodology, Investigation, Resources, Writing – original draft preparation; **NA:** Validation, Formal Analysis, Investigation, Resources; **SC:** Conceptualization, Validation, Writing – review and editing, Supervision.

Author Disclosure
The authors declared no conflict of interest.

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CASE REPORT

Recurrent Desmoid Fibromatosis of the Thyroid Gland: A Diagnostic Challenge

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Abstract

A 31-year-old Indian female with a history of near-total thyroidectomy 2.5 years prior presented with recurrent neck swelling. Magnetic resonance imaging (MRI) of the neck revealed an infiltrating mass involving the thyroid bed. Biopsy from the mass and review of slides from the previous thyroidectomy revealed a spindle cell tumour with interspersed areas of fibrosis and infiltrative edges entrapping thyroid follicles. Beta-catenin immunopositivity and CTNNB1 mutation confirmed the diagnosis of fibromatosis. The case is being reported for its rarity and the discussion of its differential diagnoses.

Key words: thyroid, fibromatosis, immunohistochemistry, molecular, thyroid nodule

INTRODUCTION

A thyroid nodule in a middle-aged euthyroid female is commonly due to colloid nodule, adenoma, nontoxic multinodular goiter or differentiated thyroid cancer. Recurrent thyroid swelling following surgery, size >4 cm, firm consistency, fixation to adjacent tissues, cervical lymphadenopathy and vocal cord paralysis indicate a probable malignant pathology.[1] We discuss a case of recurrent thyroid bed swelling in a 31-year-old Indian female with clinical symptoms suggestive of malignancy. However, histopathological and molecular examination revealed a diagnosis of desmoid fibromatosis involving the thyroid gland. The tumor is rare and a great imitator in this location which posed a unique diagnostic challenge.

CASE

A 31-year-old Indian female presented with left-sided neck swelling, rapidly increasing in size from 2 cm to 8 cm over six months. There were no associated symptoms of pain, anorexia, weight loss, difficulty in breathing or swallowing, or any features suggestive of hypo- or hyperthyroidism. Examination of the neck revealed an 8 cm x 7 cm, irregular, indurated, non-tender mass with ill-defined margins on the left anterior aspect of the neck and is fixed to the underlying structures.

The patient had a history of near-total thyroidectomy done 2.5 years ago for a left thyroid tumor. Neck enlargement had been present for one year before surgery. Ultrasound examination done at that time revealed a heterogeneous mass involving the left thyroid lobe, having significant vascularity but lacking calcifications. A near-total thyroidectomy was performed due to the presence of adhesions with underlying structures. Histopathologic examination was suggestive of Riedel's thyroiditis.

Magnetic resonance imaging of the neck done at the time of recurrence revealed an infiltrating soft tissue mass measuring 7.4 cm x 6.9 cm involving the left side of the neck extending into paravertebral space, encasing the left carotid artery and compressing the left jugular vein (Figure 1). The differential diagnoses of anaplastic thyroid carcinoma and thyroid lymphoma were also considered due to the clinical presentation. Fine needle cytology was hypocellular. Few hypo- and moderately cellular dense collagenous stromal fragments were noted. The cells were oval to spindle-shaped and had fine nuclear chromatin. The material was considered inadequately representative. A core needle biopsy showed a hypocellular spindle cell lesion. Images, sections and paraffin blocks of the previous thyroidectomy specimen were reviewed.

Grossly, the specimen was distorted, replaced entirely by the tumor, and measured 8 cm x 7 cm x 6.5 cm (Figure 2). Microscopic examination revealed a tumour composed of fibroblasts with intervening collagen deposition. The cells lacked atypia and mitotic activity. Few thin and thick-walled focally ectatic blood vessels were interspersed. The

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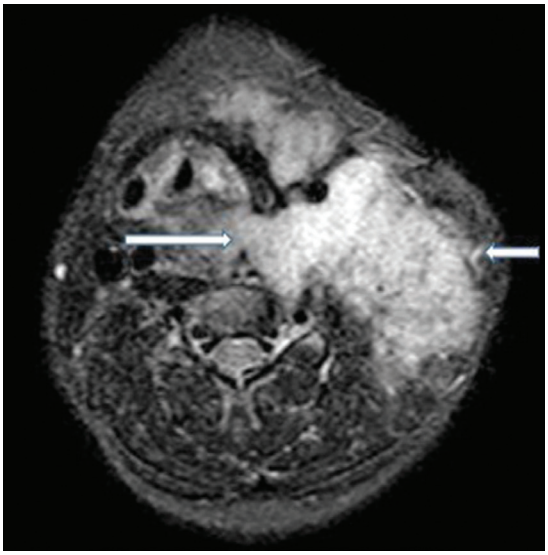

Figure 1. T2-weighted magnetic resonance image of the neck in axial view showed a hyperintense mass on the left side of the neck, extending to pre-laryngeal and prevertebral spaces *(arrows)*.

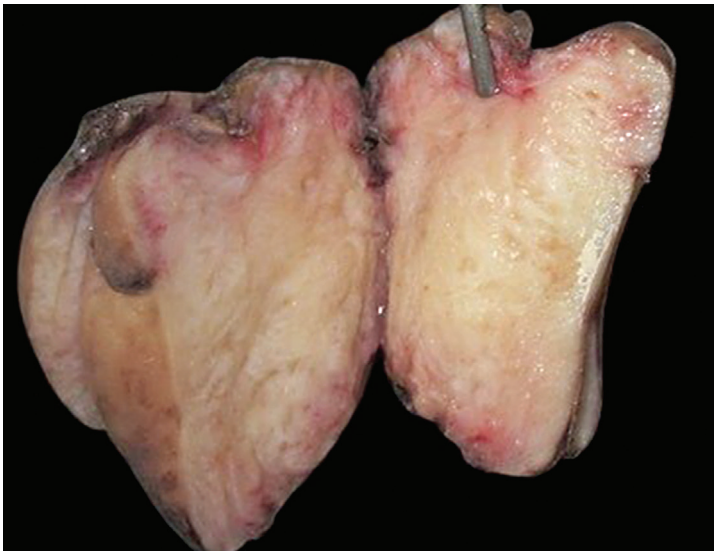

Figure 2. Gross examination of the thyroidectomy specimen showed a tumor measuring 8 cm x 7 cm x 6.5 cm, replacing the entire gland. No normal thyroid parenchyma was discernible. The cut surface appeared fleshy, grey-white in color with whorls and myxoid foci.

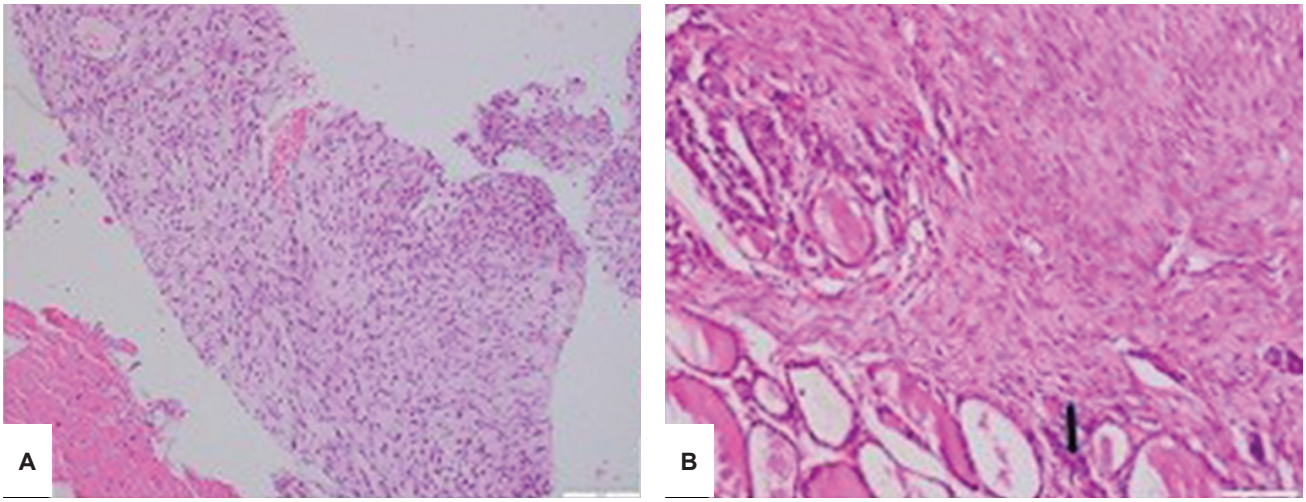

Figure 3. Microscopic examination of core biopsy *(H&E, 100x)*. **(A)** revealed a low-grade spindle cell tumour having areas of fibrosis. Examination of the thyroidectomy specimen *(H&E, 200x)*. **(B)** showed that the tumor infiltrated the normal thyroid parenchyma at the periphery *(black arrow)*.

tumour infiltrated the residual thyroid, which was within normal histological limits, and neck skeletal muscle bundles (Figure 3). Minimal focal lymphoplasmacytic infiltrate was noted within and at the advancing edge of the lesion. There was no evidence of malignancy or obliterative vasculitis. Immunohistochemical studies demonstrated that the cells were positive for smooth muscle actin (Scytek, 1A4, A00002) and desmin (Scytek, D33, A00007), and showed nuclear and cytoplasmic staining with β-catenin (BD Biosciences, 14/Beta-Catenin, 610154), but were negative for pan-cytokeratin (Bio SB, AE1 & AE3, BSB 5433), CD34 (Spring Bioscience, QBEnd/10, E1281), and S-100 (Bio SB, 4C4.9, BSB 5919) (Figure 4). Ki-67 (Invitrogen, SP6, MA5-14520) labelling index was 7 to 8% in areas of highest proliferation. Bi-directional Sanger sequencing performed on DNA extracted from the tumor block revealed a mutation involving the beta-catenin (*CTNNB1*) gene (c.134C>T; p.S45F) (Figure 5). A final diagnosis of desmoid fibromatosis involving the thyroid gland was rendered.

Surgery was not attempted as the mass was encasing vital neck structures. The patient was placed on Sorafenib, a tyrosine kinase inhibitor. On a follow-up magnetic resonance imaging (MRI) at two months, there has been no change in the extent of the tumor suggesting stable disease.

DISCUSSION

Desmoid fibromatosis is a locally aggressive deep-seated proliferation of fibroblastic/ myofibroblastic cells. It has a high propensity for local recurrence but lacks metastatic potential. Extremities are the most common sites for this

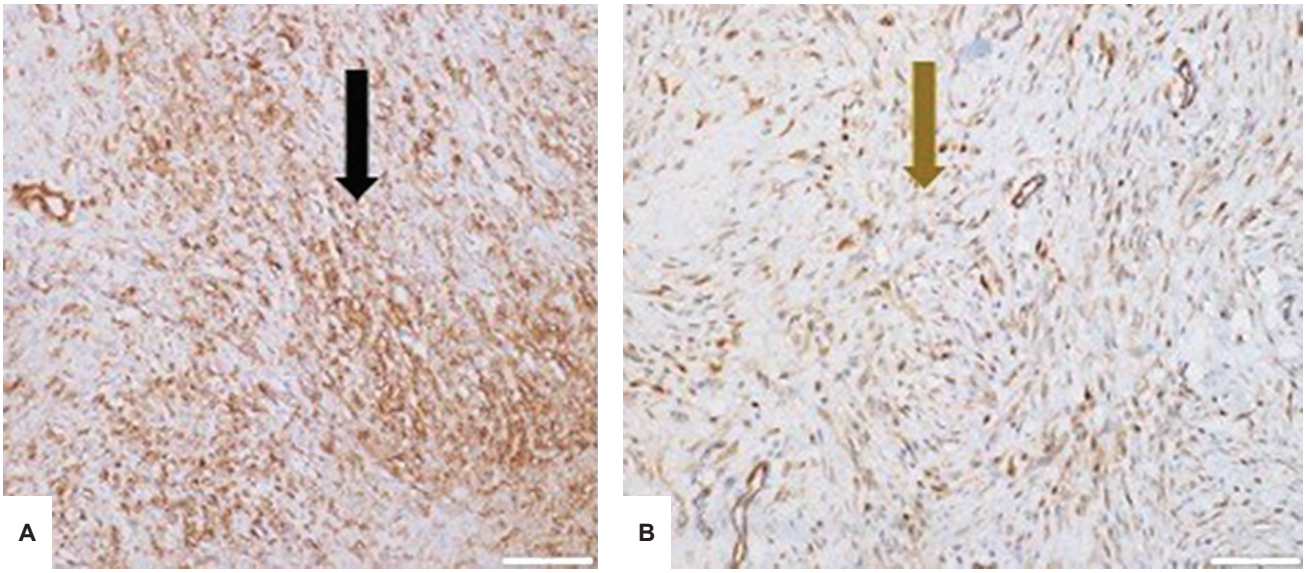

Figure 4. Immunohistochemical staining of the tumor cells showed **(A)** positivity for smooth muscle actin *(black arrow; IHC, 200x)* and **(B)** nuclear and cytoplasmic expression of β-catenin *(brown arrow; IHC, 200x)*.

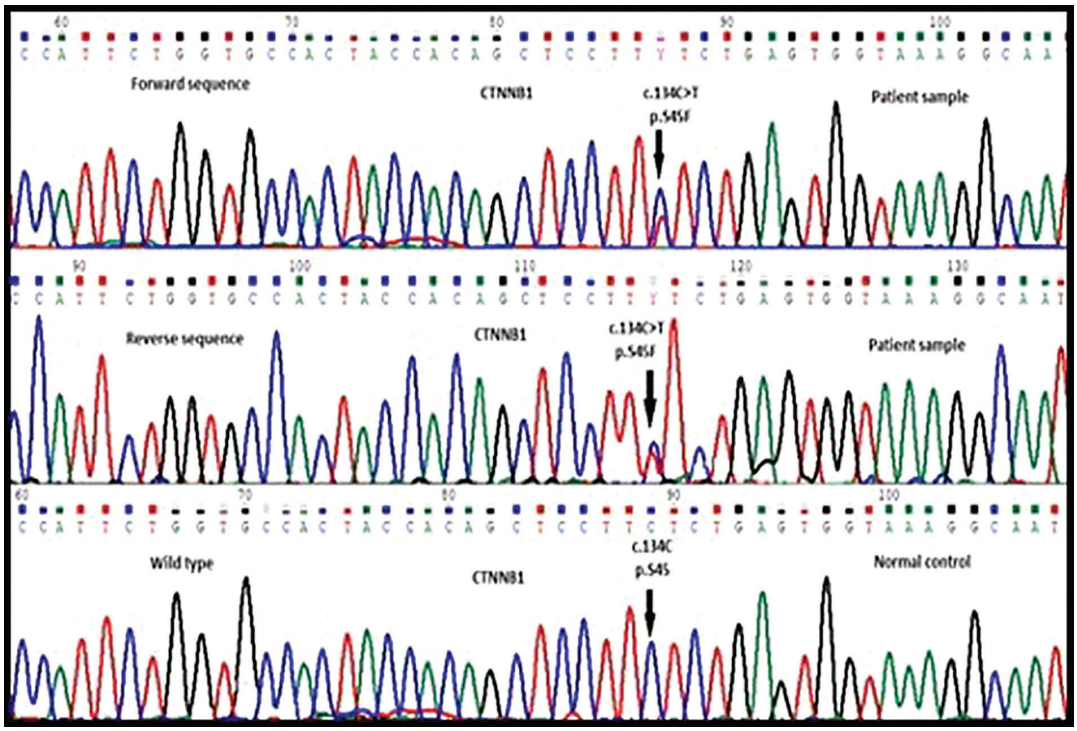

Figure 5. Bi-directional Sanger sequencing performed on DNA extracted from the tumor block revealed a mutation at codon 45 of exon 3 (c.134C>T; p.S45F) of the β-catenin (CTNNB1) gene *(arrows)*.

condition, accounting for 30 to 40% of cases. Head and neck involvement is rare, occurring in 7 to 15%.[2] Thyroid disease is even rarer, with only six cases described in five reports.[3-7] The Department of Pathology of the All India Institute of Medical Sciences, New Delhi, receives an average of about 250 thyroidectomy specimens per year. The patient is the first documented case since 2014. As highlighted in the present report, the atypical site of involvement and differential diagnoses with other more common primary lesions posed a diagnostic challenge leading to a delay in appropriate therapy.

Table 1 summarizes the details of all the cases reported to date.[3-7] The disease shows a female predilection. The age at presentation has ranged from the third to the seventh decade.[3-7] The patients may present with thyroid swelling, associated with compression symptoms like dysphonia, dyspnea and dysphagia.[4,5] History of injury with a wire was reported in one of the two cases reported by Schwarzlmüller.[3] The preoperative diagnoses were varied, including thyroid carcinoma, follicular neoplasm, multinodular goitre[3] or spindle cell lesion.[3-7] Management was surgical in all. In one patient, owing to the fixation of the mass to the trachea

Table 1. Summary of cases with thyroid fibromatosis reported in literature

Author, year	No. of cases	Age/Sex	FNAC	Molecular analysis	Management	Follow-up
Current case	1	31/F	Few collagenous stromal fragments	CTNNB1 p.S45F c.134C>T	Total thyroidectomy (at first presentation), TKI (at recurrence)	Recurrence 2.5 years after first surgery
Sinha, 1998[4]	1	26/F	ND	ND	Limited surgery to relieve tracheal compression	Not mentioned
Samsi, 1992[5]	1	60/M	Spindle cell tumor	ND	Near total thyroidectomy	No recurrence after 1 year of follow-up
Schwarzlmüller, 1978[3]	2	38/F	ND	ND	Extent of resection not detailed	Recurrence 2 and 4 years after first surgery
		68/F	ND	ND	The extent of resection is not detailed	Not mentioned
Simões-Pereira, 2016[6]	1	63/F	Colloid goiter	ND	Total thyroidectomy	Developed vocal cord paralysis after surgery; no recurrence till 11 years of follow-up
Mehdizadeh, 2020[7]	1	37/M	Follicular neoplasm	ND	Near total thyroidectomy	No recurrence after 2 years of follow-up

Abbreviations: FNAC, fine needle aspiration cytology; F, female; M, male; ND, not done; TKI, tyrosine kinase inhibitor

and encasement of the major vessels, only a limited surgery to relieve tracheal compression could be performed.[4] Of the remaining patients with available follow-up, one developed recurrence twice, 2 and 4 years after initial surgery; and one had a recurrence-free follow-up of 11 years.[3,7]

Desmoid-type fibromatosis commonly harbors sporadic activating mutations of the *CTNNB1* gene involving the codons 41 and 45 of exon 3. Inherited cases arising in the setting of Gardner syndrome have germline *APC* gene mutations. In both situations, there is nuclear accumulation of β-catenin protein detectable by immunohistochemistry.[2] Other etiologies include an estrogen-rich environment and previous trauma such as prior surgery.[2]

A recent review by Zhang et al. discusses the therapeutic modalities for fibromatosis.[8] The treatment strategies include a watch-and-wait strategy, surgery, radiotherapy, chemotherapy, hormonal therapy, non-steroidal anti-inflammatory drugs, high-intensity focused ultrasound, and ablation techniques.[8] However, none of these modalities is superior in preventing local recurrences.[8]

Watch-and-wait strategy is preferred for asymptomatic patients with head and neck fibromatosis, as 20 to 30% undergo spontaneous regression.[9] Patients with progressive disease should initially be managed with anti-estrogenic hormonal therapy.[9] It is, however, slow to act and higher doses are usually poorly tolerated by young females.[9] Chemotherapy is considered for patients failing hormonal therapy. Surgery is recommended for patients not responding to hormonal and chemotherapeutic agents, but only if resection is possible.[9,10] Younger patient age and tumor properties, including extra-abdominal location, larger size, and *CTNNB1* p.Ser45Phe mutation, have been associated with local recurrence after surgery.[2] Exclusive radiotherapy or surgery followed by radiotherapy should be considered when it is not feasible to perform R0 resection in critical areas like the head and neck.[9] All the cases reported in literature have been managed surgically.

In our case, surgery was performed at the time of initial presentation. The patient had recurrence 2.5 years post-surgery with rapid disease progression. Extensive local invasion precluded surgery; hence, sorafenib, a tyrosine kinase inhibitor was started. The patient is on regular clinical and radiologic follow-up. A phase III trial evaluating sorafenib in 87 patients with progressive, symptomatic or recurrent fibromatosis revealed a 2-year progression-free survival rate of 81% compared to 36% in patients given a placebo.[11]

The cornerstone for the diagnosis of desmoids is histopathology. They show an infiltrative proliferation of fibroblasts lacking atypia. Other primary thyroid lesions which are more common and show prominent fibroblast proliferation include Riedel's thyroiditis, the fibrous variant of Hashimoto thyroiditis; papillary thyroid carcinoma (PTC) with fibromatosis/fasciitis-like stroma; and post-radiation fibrosis.

Both Riedel's thyroiditis and fibromatosis clinically mimic malignancy. Awareness, a high index of suspicion and careful pathological evaluation including immunohistochemistry and molecular analysis, help in resolving the diagnostic dilemma. Both Riedel's thyroiditis and fibromatosis clinically and morphologically show extensive fibrosis of thyroid parenchyma extending into adjoining neck structures. The presence of prominent IgG4-positive plasma cell infiltrates, obliterative phlebitis and lack of nuclear expression of β-catenin favor a diagnosis of Riedel's thyroiditis.[12] The fibroinflammatory process in Hashimoto's thyroiditis is limited to but affects the entire thyroid gland, in contrast to Riedel's thyroiditis. Additional features in the former include the presence of oncocytic metaplasia and lymphoid germinal centres. Another important differential diagnosis is PTC with fibromatosis/fasciitis-like stroma, which is also a rare pathology, comprising up to 0.5% of all cases of PTC. Histologically, it is a biphasic tumor. Myofibroblastic proliferation morphologically similar to fibromatosis or nodular fasciitis is noted along with a variable proportion of PTC. Like fibromatosis, the mesenchymal component shows *CTNNB1* mutation while the epithelial component commonly harbors *BRAF* V600E mutation.[13] As the carcinoma may form only a minor component of the entire tumor, it may be absent on preoperative cytology

evaluation. An extensive sampling of the resected specimen is essential to identify the PTC component.

CONCLUSION

Fibromatosis involving the thyroid gland is a rare disease and poses a diagnostic challenge for the treating clinician and the pathologist. It presents as a locally invasive thyroid mass and depending upon the extent of the disease may be misdiagnosed as multinodular goiter, thyroid malignancy or Riedel's thyroiditis. A high index of suspicion and multi-departmental coordination are essential to reach a correct diagnosis at the time of initial presentation and workup.

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

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

CRediT Author Statement
BKS: Conceptualization, Investigation, Writing – original draft preparation**. SC:** Conceptualization, Writing – original draft preparation. **YSR:** Conceptualization, Writing – review and editing. **SA:** Conceptualization, Writing – review and editing. **SR:** Conceptualization, Writing – review and editing. **VS:** Conceptualization, Writing – review and editing, Supervision.

Author Disclosure
The authors declared no conflict of interest.

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CASE REPORT

Journal of the ASEAN Federation of Endocrine Societies

New-onset Thyroid Eye Disease after COVID-19 Vaccination in a Radioactive Iodine-Treated Graves' Disease Patient: A Case Report and Literature Review

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Abstract

Autoimmunity associated with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been well-described as the mechanism of development of thyroid dysfunction following Coronavirus Disease 19 (COVID-19) infection and SARS-CoV-2 vaccination. However, the occurrence of thyroid eye disease (TED) after SARS-CoV-2 vaccination is scarcely described. The postulated mechanisms include immune reactivation, molecular mimicry and the autoimmune/inflammatory syndrome induced by adjuvants (ASIA). We report a case of new-onset TED after receiving the SARS-CoV-2 vaccine.

Key words: thyroid eye disease, SARS-CoV-2 vaccine, radioactive iodine therapy, autoimmune/inflammatory syndrome induced by adjuvants, molecular mimicry

INTRODUCTION

Since COVID-19 struck the world, as of December 12, 2022, there have been 645,084,824 people affected, and it has claimed more than 6 million lives.[1] SARS-CoV-2, the virus responsible for the disease, has been shown to cause a multitude of systemic disorders including immune dysregulation, such as autoimmune thyroiditis or Graves' disease (GD).[2] To address the COVID-19 pandemic, vaccination against COVID-19 was started in December 2020, and an estimated total of 13 billion doses have been administered by the end of 2022.[1] While COVID-19 vaccination has successfully reduced the number of cases and the severity of the disease, there have been many cases of new-onset or relapse of GD and subacute thyroiditis following COVID-19 vaccination reported.[3-11] However, there are limited reports of thyroid eye disease (TED) after COVID-19 vaccination. We describe a patient with underlying GD who developed TED three weeks after injection of BNT162b2 (Pfizer-BioNTech) mRNA COVID-19 vaccine.

CASE

A 54-year-old, non-smoking, Chinese male with underlying Kallman Syndrome and Type 2 Diabetes Mellitus (T2DM) was diagnosed with GD without TED in 2003. He was given carbimazole for three years and achieved remission in 2006. He had relapsed with subclinical hyperthyroidism after 11 years in April 2017 with a thyroid stimulating hormone (TSH) level of <0.01 mIU/L (0.35-4.94) and a free T4 (fT4) level of 18.8 pmol/L (9-19.05). As a result, carbimazole was restarted but discontinued a year later. He remained clinically and biochemically euthyroid until June 2019 when he became overtly hyperthyroid again with a suppressed TSH of <0.01 mIU/L and elevated fT4 of 29.73 pmol/L. He subsequently underwent radioactive iodine (RAI) therapy in September 2020. Two months later, he developed hypothyroidism and was started on levothyroxine replacement. He achieved euthyroidism with levothyroxine 150 mcg daily in June 2021 (7 months after levothyroxine replacement therapy initiation) with TSH of 0.36 mIU/L (0.35-4.94) and fT4 of 11.29 pmol/L (9-19.05). His baseline photograph prior to the vaccine is seen in Figure 1A.

On the months of July and August 2021, he received his first and second doses of BNT162b2 (Pfizer-BioNtech) mRNA COVID-19 vaccine, respectively. Three weeks after receiving the second dose of the vaccine, he experienced new-onset bilateral eyes redness, dryness, proptosis and diplopia, which were gradually worsening (Figure 1B). He has never contracted COVID-19 before the reactivation of the hyperthyroidism to the time of RAI therapy and before the onset of TED. There were also no other acute infections or recent surgery. He sought treatment at a private ophthalmology clinic in November 2021 when his eye condition became worse. He underwent magnetic

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resonance imaging (MRI) of the orbits which showed bilateral extraocular muscle enlargement, especially of the inferior and medial rectus muscle (Figure 2) and proptosis. He was managed as TED for which he was given weekly intravenous (IV) methylprednisolone (MTP) 500 mg for 4 weeks, followed by 750 mg weekly for 2 weeks. His TED improved after the treatment. Unfortunately, he defaulted to subsequent follow-up.

In March 2022, his TED worsened, presenting with bilateral exophthalmos, chemosis, conjunctival injection, swollen eyelids and caruncles (Figure 1C). He was assessed to have active moderate-to-severe TED with a clinical activity score (CAS) of 4 out of 7. On further ophthalmologic assessment, his vision was intact, but there was diplopia on the upward gaze and secondary ocular hypertension. He had normal TSH, fT4 and fT3 levels (0.46 mIU/L [0.35-4.94], 16.47 pmol/L [9-19.05] and 4.5 pmol/L [2.6-5.7], respectively). However, the TSH-receptor antibodies (TRAb) level was elevated at 3.60 IU/L (<1.75) and anti-thyroid peroxidase (TPO) antibodies of >600 IU/ml (0-34). Unfortunately, there were no baseline auto-antibody tests for comparison.

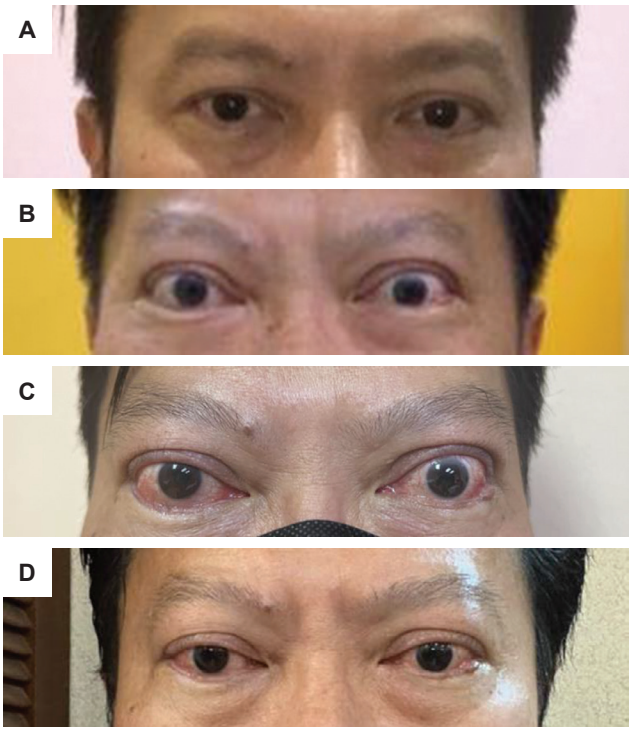

Figure 1. (A) Patient's photography *(taken with the patient's cellphone)* in April 2021 before BNT162b2 mRNA COVID-19 vaccine indicating no obvious sign of clinical thyroid eye disease. **(B)** Patient's photograph 3 weeks after the second dose of the vaccine indicating exophthalmos, eyelid swelling and upper eyelid retraction. **(C)** Clinical photograph of the patient at presentation in March 2022, demonstrating bilateral exophthalmos, chemosis, conjunctival injection, upper eyelid retraction, swollen eyelids and caruncles. **(D)** Patient's photography after third dose of IV methylprednisolone indicating marked improvement of the eye signs with no eyelid and caruncles swelling and chemosis, albeit with mild conjunctival injection.

Intravenous MTP 500 mg was restarted and given weekly for 6 doses, followed by 200 mg weekly for another 6 doses. Azathioprine was started simultaneously. Congestive eye symptoms and diplopia significantly improved after the third dose of MTP (Figure 1D), with a CAS of 1 out of 10 attributable to mild conjunctival injection.

DISCUSSION

Thyroid eye disease (TED) is one of the extrathyroidal manifestations of autoimmune thyroid disease resulting from an autoimmune and inflammatory process. It is relatively rare, with females more commonly affected than males, and moderate-to-severe forms accounting 5 to 6% of cases.[12] Risk factors for developing TED include smoking, thyroid dysfunction, high serum level of thyrotropin receptor antibodies, RAI treatment, and hypercholesterolemia.[12] While there are limited data comparing prevalence rates of GD and TED among different ethnic groups within populations, a meta-analysis and systematic review by Chin et al., reported that the pooled prevalence for thyroid eye disease was 44% in Asia, 38% in Europe and 27% in North America.[13]

Since the start of the COVID-19 pandemic, SARS-CoV-2-related thyroiditis has been increasingly recognized. Lui et al., reported that 15% of patients with mild to moderate COVID-19 had thyroid dysfunction, and SARS-CoV-2 could potentially exacerbate pre-existing autoimmune thyroid disease.[14] There were also numerous reported cases of thyroid dysfunction due to new-onset or relapse of GD or subacute thyroiditis following the SARS-CoV-2 vaccination described in recent literature.[3-11]

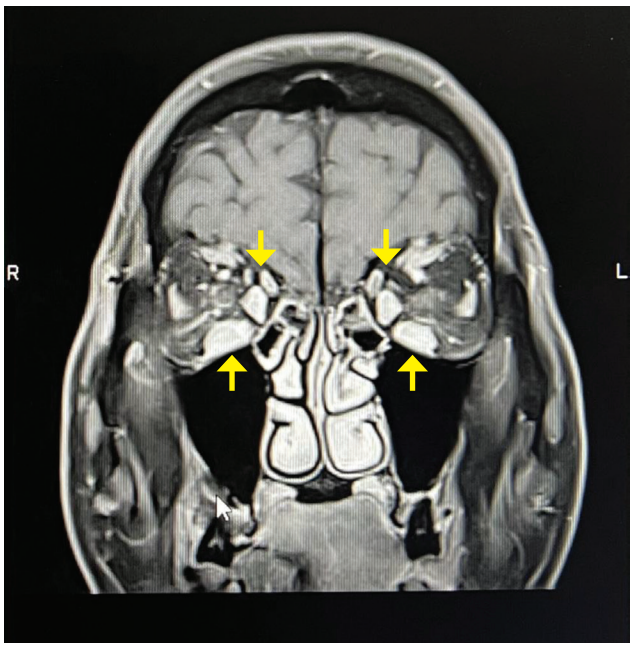

Figure 2. Coronal MRI of the orbits demonstrating bilateral extraocular muscle enlargement especially the inferior and medial rectus muscles *(yellow arrows)* consistent with thyroid eye disease.

In contrast, TED after SARS-CoV-2 vaccination is rare. To date, there have been 16 cases of reactivation or new-onset TED after SARS-CoV-2 vaccination reported.[10,15-20] A summary table (Table 1) is presented comparing the patient characteristics, clinical presentation and treatments of other similar post-vaccination TED patients together with the index patient of this report.

The mechanisms of developing TED were postulated to be similar to how GD occurs after vaccination. The BNT162b2 (Pfizer-BioNtech) mRNA COVID-19 vaccine induces spike protein-specific neutralizing antibodies associated with protective immunity.[21] Vojdani et al., conducted a study in vivo and found that the SARS-CoV-2 spike protein, nucleoprotein, and membrane protein all cross-reacted with TPO, and many TPO peptide sequences shared homology or similarity with sequences in various SARS-CoV-2 proteins.[22] These findings suggest that the SARS-CoV-2 vaccine may lead to the onset of autoimmune thyroid disease and TED via molecular mimicry between the SARS-CoV-2 spike proteins and thyroid proteins or TPO peptides.[22] The antibodies against these viral targets may also cause thyroid tissue damage, leading to the release of further auto-antigens and the potential development of other auto-antibodies that may trigger TED, such as thyroid stimulating immunoglobulin (TSI), TRAb or anti-TPO.[19,23,24] This could explain the mechanism for this patient case, and in the 12 other patients out of the 16 reported cases who did not undergo total thyroidectomy. The four patients who underwent total thyroidectomy might have remnant thyroid tissue that could have served as an immune target. Also, it is important to note that our patient had both elevated TRAb and anti-TPO, whereas most of the reported cases had only either raised TRAb or TSI alone.

Another postulated mechanism was autoimmune/inflammatory syndrome induced by adjuvants (ASIA). ASIA is an entity that incorporates diverse autoimmune conditions induced by exposure to various adjuvants that are found in many vaccines.[25] Adjuvants are substances that can trigger autoimmunity via a variety of mechanisms, such as alteration of the host's immune system, polyclonal activation of B cells, effects on cellular immunity, immunoregulatory cells, viral-induced antibodies and acceleration of molecular mimicry.[25] These, in turn, lead to the promotion of inflammation through the activation of macrophages and fibroblasts, as well as the production of Th-1 cells and adipocyte differentiation, which are similar mechanisms seen in TED.[26] In mRNA vaccines, polyethylene glycol (PEG) in conjugation with lipid nanoparticles may act as an adjuvant to trigger an autoimmune reaction following SARS-CoV-2 vaccination. Coincidentally, the reported TED cases and our case were given mRNA vaccines.

Despite the myriad of individuals who received SARS-CoV-2 vaccination, autoimmune thyroid diseases, such as GD and TED, are still relatively rare or underreported. It is hypothesized that these autoimmune conditions may commonly occur in genetically susceptible individuals, where the T lymphocytes are excessively sensitized to the TSH receptor thereby causing GD or TED.[20] It is also possible that epigenetic changes or alterations in the patient's microbiome, such as those demonstrated following mRNA SARS-CoV-2 vaccination, may play a role in TED pathogenesis.[19] Lastly, Sriwijitalai and Wiwanitkit suggested that vaccination-induced increase in blood viscosity is another possible pathophysiological process.[27] Vaccines can significantly increase blood viscosity, and very high blood viscosity is associated with exophthalmos at a stable stage of hyperthyroidism.[27]

The time of the onset of TED after mRNA SARS-CoV-2 vaccination in the reported cases ranged from day 1 to day 60 following the first or second dose of vaccination (Table 1), whereas the symptoms onset of thyroid dysfunction ranged from 2 to 37 days after SARS-CoV-2 vaccination.[10,11] In our case, the TED symptoms started manifesting after 3 weeks from the second SARS-CoV-2 vaccine and approximately one year after RAI treatment.

The study by Traisk et al., reported that RAI treatment is a significant risk factor for the development of TED in Graves' hyperthyroidism.[28] The proportion of worsening or development of TED after 1 year was 31% in patients who received RAI therapy compared to 16% who received methimazole.[28] While the study by Kung et al., reported that the mean time for development or exacerbation of TED after RAI was 6.7 ± 2.2 months (range, l-15 months).[29] The temporal relationship suggests that either the SARS-CoV-2 vaccine or RAI could be the triggering event in our patient or the presence of both might have amplified the autoimmune and inflammatory cascades leading to TED. It was also found that hypothyroidism with elevated TSH is an important adverse factor for the development or exacerbation of TED. The adjunctive use of methimazole after RAI was unable to prevent the development or exacerbation of TED.[29] In our case, the patient has already achieved a euthyroid state at the time of TED manifestation. Other authors also reported that their patients had received RAI treatment several years before and were already euthyroid before the onset of TED.[15-17]

Ten of the reported TED cases had a history of being treated for TED and were stable prior to the administration of SARS-CoV-2 vaccine. This contrasts with the other cases which documented new-onset TED. Our patient received a total of 12 doses of IV MTP, which afforded significant improvement of the patient's eye symptoms after the third dose of the second cycle. Azathioprine was added simultaneously during the second cycle of MTP. Azathioprine has been shown to reduce the relapse rate after glucocorticoid withdrawal.[12] Among the other 16 reported cases, the clinical presentations ranged from mild, moderate-to-severe to sight-threatening disease, with the milder disease seen mostly in new-onset TED cases. Generally, most patients with mRNA SARS-CoV-2 vaccine-associated TED had a favorable response to

Table 1. Reported cases of thyroid eye disease after COVID-19 vaccination

	Country	Age / Sex	Smoking status	Type of vaccine/dose	Post-vaccination symptoms onset	History of GD / TED	Pre-vaccination thyroid status	History of RAI	History of total thyroidectomy	Laboratory test results at diagnosis					CAS	Severity of TED	Rx for TED	Symptoms improvement
										TSH (RR)	fT4 (RR)	fT3 (RR)	TRAb (RR)	TSI (RR)				
Our case	Malaysia	54 / male	N	mRNA Pfizer / 2nd	3 weeks	Y / N	Euthyroid with LT	Y (1 year ago)	N	0.46 (0.35-4.94 mIU/L)	16.47 (9-19.05 pmol/L)	4.5 (2.6-5.7 pmol/L)	3.6 (<1.75 IU/L)	NA	4/7	Moderate-to-severe	MTP	Yes (after 3rd infusion)
Case 1[10]	United States	51 / female	N	mRNA Pfizer / 2nd	4 days	N / N	Euthyroid	N	N	<0.01 (0.27-4.2 mIU/L)	3.72 (0.93-1.7 ng/dL)	12.6 (2-4.4 ng/dL)	5.04 (<1.5 IU/L)	NA	3/7	Mild	Thyroidectomy	Yes
Case 2[15]	United States	50 / female	N	mRNA Pfizer / 2nd	3 days	Y / N	Euthyroid with LT	Y (12 years ago)	N	Normal	Normal	Normal	NA	2.29 (<0.55 IU/L)	5/7	Moderate-to-severe	Teprotumumab	Yes (after 2nd infusion)
Case 3[16]	United States	66 / female	N	mRNA Moderna / 2nd	3 weeks	Y / Y*	Euthyroid with LT	Y (15 years ago)	N	0.04 (0.3-5.0 uIU/mL	1.7 (0.7-1.7 ng/dL)	NA	5.51 (<1.5 IU/L)	3.91 (<0.55 IU/L)	6/10	Moderate-to-severe	Teprotumumab	Yes (at 5 months)
Case 4[16]	United States	53 / female	N	mRNA Pfizer / 1st	1 day	N / N	Euthyroid	N	N	0.99 uIU/mL (0.3-5.0 uIU/mL	0.9 (0.7-1.7 ng/dL)	NA	NA	3.21 (<0.55 IU/L)	NA	Moderate-to-severe	Teprotumumab	Yes (at 8 months)
Case 5[16]	United States	45 / female	N	mRNA Moderna / 1st	3 weeks	Hashimoto / Y*	Euthyroid with LT	N	N	Abnormal	Abnormal	NA	NA	NA	NA	Mild-to-moderate	No Rx	Yes
Case 6[17]	Italy	58 / female	NA	mRNA Pfizer / 2nd	3 days	Y / Y*	Euthyroid with LT	Y (2 years ago)	N	1.17 (0.4-4.00 mIU/L)	1.26 (0.7-1.7 ng/dL)	3.54 (2.7-5.7 ng/dL)	6.82 (<1.5 IU/L)	NA	6/10	Moderate-to-severe	Teprotumumab	NA
Case 7[17]	Italy	43 / male	NA	mRNA Pfizer / 1st	2 weeks	Y / Y*	Euthyroid with MMI	N	N	2.316 (0.4-4.00 mIU/L)	0.96 (0.7-1.7 ng/dL)	3.4 (2.7-5.7 ng/dL)	20.7 (<1.5 IU/L)	NA	NA	Sight-threatening disease	NA	NA
Case 8[18]	United States	51 / female	Former smoker	mRNA Moderna / 2nd	2 weeks	Y / Y*	NA	NA	NA	NA	NA	NA	NA	NA	9/10	NA	Prednisolone, teprotumumab, orbital decompression	Yes (13 months after teprotumumab, 2 months after surgery)
Case 9[19]	United States	50 / male	N	mRNA Pfizer / 2nd	3 weeks	Y / Y*	Euthyroid with LT	N	Y	2.3 mIU/L	NA	NA	NA	4.46 (<1.75 IU/L)	7/10	Moderate-to-severe	MTP, tocilizumab and teprotumumab	Yes (after 3rd teprotumumab infusion)
Case 10[19]	United States	71 / female	N	mRNA Moderna / 2nd	3 days	Hypothyroidism / N	Euthyroid with LT	N	N	Undetectable	1.4 (0.93-1.70 ng/dL)	3.9 (2.3-4.2 ng/dL)	NA	5.5 (≤1.3)	4/7	Moderate-to-severe, progressed to sight-threatening disease	MTP followed by teprotumumab	Yes (after 3rd teprotumumab infusion)
Case 11[20]	France	70 / female	NA	mRNA Pfizer / 2nd	60 days	Y / Y*	Euthyroid with LT	N	Y	1.65 mIU/L	20 pmol/l	NA	>40 IU/L	NA	4/7	Moderate-to-severe	Prednisolone, tocilizumab	Yes (after 2 weeks of tocilizumab infusion)
Case 12[20]	France	43 / male	NA	mRNA Moderna / 1st	1 day	Y / Y*	Mild hypothyroid with CBZ	N	N	4.04 mIU/L	6.2 pmol/l	NA	Absent	NA	7/7	Sight-threatening disease	Tocilizumab	Yes (after 1st infusion)
Case 13[20]	France	73 / male	NA	mRNA Pfizer / 1st	21 days	Y / N	Euthyroid with CBZ	N	N	2.4 mIU/L	NA	NA	Normal	NA	3/7	Mild	Selenium,MTP	Yes (after 1st infusion
Case 14[20]	France	45 / female	NA	mRNA Moderna / 2nd	NR	Y / Y*	Euthyroid with LT	N	Y	0.76 mIU/L	NA	NA	151 IU/L	NA	4/7	Moderate-to-severe	Lubricants	Yes (at 5 months)
Case 15[20]	France	48 / male	NA	mRNA Moderna / 2nd	30 days	Y / Y*	NR	N	Y	<0.01 mIU/L	21 pmol/l	NA	28 IU/L	NA	5/7	Sight-threatening disease	MTP, orbital decompression, teprotumumab	Yes (after 1st infusion)
Case 16[20]	France	39 / female	NA	mRNA Pfizer / 1st	7 days	N / N	Euthyroid	N	N	0.3 mIU/L	NA	NA	5 IU/L	NA	2/7	Mild	Selenium	Unchanged

GD: Graves' disease; TED: thyroid eye disease; TSH: thyroid-stimulating hormone; fT4: free thyroxine; fT3: free triiodothyronine; TRAb: TSH receptor antibody; TSI: thyroid stimulating immunoglobulin; CAS: Clinical activity score; MMI: methimazole; CBZ: carbimazole; LT: levothyroxine; MTP: Methylprednisolone; NA: not available; Y: yes; N: no; RR: reference range.
*Stable disease after receiving Rx for TED

teprotumumab, including patients with the sight-threatening disease.[19,20] Three cases were given oral prednisolone, MTP and a combination of MTP and tocilizumab with a limited response, but symptoms improved favorably after teprotumumab treatment.[18,19] While teprotumumab has been proven effective in TED treatment, its use is restricted by cost and availability, and long-term efficacy and safety data are still lacking.[12]

CONCLUSION

In conclusion, to the best of our knowledge, our patient is the first reported case of mRNA SARS-CoV-2 vaccine-associated TED reported in Asia. Although the temporal relationship of developing TED after COVID-19 vaccination might be suggestive, other possible factors may be contributory, such as prior RAI treatment in our case. While there is no cure for COVID-19 yet, the vaccines have been instrumental in its prevention and control. By and large, the benefits of the SARS-CoV-2 vaccine outweigh the risks. Patients with known autoimmune thyroid disease should be monitored closely and periodically after the SARS-CoV-2 vaccination as they might develop TED and require prompt treatment to alleviate the symptoms and signs. Finally, further studies are required to identify the potential mechanisms of new-onset or reactivation of TED following the administration of mRNA SARS-CoV-2 vaccines and to understand the possibility of ethnicity-related predisposition.

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Ethical Consideration
Informed consent has been taken before submission of the manuscript.

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

CRediT Author Statement
JHIT: Conceptualization, Methodology, Software, Validation, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **NM:** Conceptualization, Writing – review and editing, Supervision, Project administration; **NW:** Conceptualization, Writing – review and editing, Visualization, Supervision, Project administration.

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IMAGES IN ENDOCRINOLOGY

Multiple Xanthoma Tuberosum in a Case of Familial Homozygous Hypercholesterolemia

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Key words: xanthoma, familial, hypercholesterolemia, LDL

A 15-year-old, Indian, female child of a second-degree consanguineous marriage, presented with polymorphic yellowish-brown nodular cutaneous lesions over the dorsal aspect of both elbows, knees (Figure 1A) and buttocks (Figure 1B). These were suggestive of xanthoma tuberosum and were first noted at 4 years old. There were no spots over the eyelids, acanthosis, skin tags or tendon xanthomas. Arcus juvenilis was not noted. A bilateral carotid bruit was appreciated. Her father and mother also have hypercholesterolemia and were receiving atorvastatin.

Her lipid profile showed total cholesterol: 790 mg/dL, LDL-C: 736 mg/dL, HDL-C: 34 mg/dL, VLDL-C: 124 mg/dL and triglyceride: 102 mg/dL. There was also increased carotid intima media thickness on doppler and severe aortic stenosis and normal Ejection fraction of 62% on echocardiogram.

Genomic studies revealed homozygous mutation in both alleles of the LDL-R gene, associated with autosomal dominant Familial Hypercholesterolemia. The 737-position arginine (R) was also changed. This change, previously reported by Mozas et al., in a Spanish patient,[1] was not present in genomic databases reviewed as of writing.

The patient was subsequently diagnosed with Homozygous Familial Hypercholesterolemia using the Simon Broome Register criteria.[2] Diet modification, atorvastatin and ezetimibe were started, followed by subcutaneous evolocumab. After 6-8 weeks of therapy with atorvastatin, ezetimibe and evolocumab, her cholesterol levels were markedly reduced by 82 and 89%, respectively. However, the patient discontinued the use of evolocumab due to financial constraints.

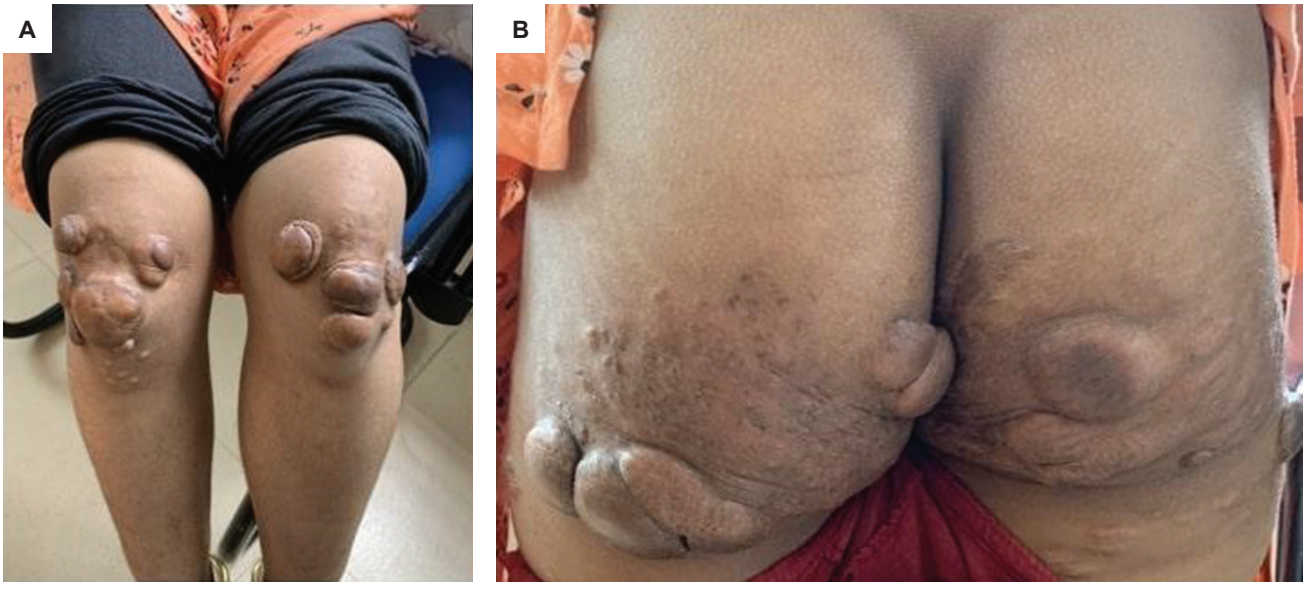

Figure 1. Large tuberous xanthomas on both knees **(A)** and buttocks **(B)**.

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Homozygous familial hypercholesterolemia is a disorder of lipoprotein metabolism from mutations in the low-density lipoprotein receptor (LDLR) gene,[3] which manifests as xanthomas.[4]

Guidelines for dyslipidaemia recommend high intensity statin treatment at 6-10 years of age, with healthy lifestyle measures and use of PCSK-9 inhibitors for children with homozygous familial hypercholesterolemia. Goals for children >10 years of age are LDL-C <3.5 mmol/l (135 mg/dL) or at least a 50% reduction from the baseline.[5, 6]

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

Statement of Authorship
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PS: Conceptualization, Methodology, Software, Validation, Investigation, Resources, Writing – review and editing, Visualization, Supervision, Funding acquisition. **PB:** Conceptualization, Formal analysis, Data curation, Writing – original draft preparation, Visualization, Project administration. **AD:** Conceptualization, Formal analysis, Resources, Writing – original draft preparation, Visualization, Funding acquisition.

Author Disclosure
The authors declared no conflict of interest.

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Journal of the ASEAN Federation of Endocrine Societies

IMAGES IN ENDOCRINOLOGY

Panhypopituitarism and Bifid Uvula

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Key words: bifid uvula, hypopituitarism, midline defect

A 20-year-old Indian male presented with abnormal thyroid function tests and poor development of secondary sexual characteristics. Perinatal history included breech delivery, delayed cry and lower respiratory tract infection. Developmental delays, below average scholastic performance and note of being the shortest child in class were reported.

Examination revealed lack of facial hair, depressed nasal bridge, low set ears, nasal speech, short stature, increased arm span, bifid uvula (Figure 1), sparse axillary and pubic hair (A0 P1), low testicular volume and short phallic length. Combined pituitary hormone deficiency was suspected.

Laboratories show low FT4 (0.62 ng/dl) with normal TSH (3.21 mIU/mL), low levels of 8AM testosterone (<20 ng/dl), LH (0.384 U/L), FSH (0.201 U/L), 8AM cortisol (<1 mcg/dl), serum IGF1 (<20 ng/ml) and repeat FT4 (0.609 ng/dl). Serum calcium, phosphorus, and vitamin D levels were normal. Radiographs showed scoliosis and 11.5–12.5 years bone age. Hypoplastic anterior pituitary with eutopic posterior pituitary bright spot (Figure 2) were appreciated on MRI.

Following guidelines for hypopituitarism,[1] he was given hydrocortisone, levothyroxine and monthly parenteral testosterone. At follow up, he reported improved sense of well-being, decreased fatigability and increased strength with noted progression of pubertal development (A0P3).

Since the hypothalamus, pituitary gland, and oral cavity develop very closely during early embryonic life, defects in one may herald abnormalities in others. Midline defects such as a bifid uvula, has been associated with hypopituitarism.[2] Breech delivery has also been shown to be associated with hypopituitarism.[3] The presence of midline defects along with other risk factors for hypopituitarism should alert physicians to the possibility of pituitary defects to facilitate earlier evaluation and intervention.

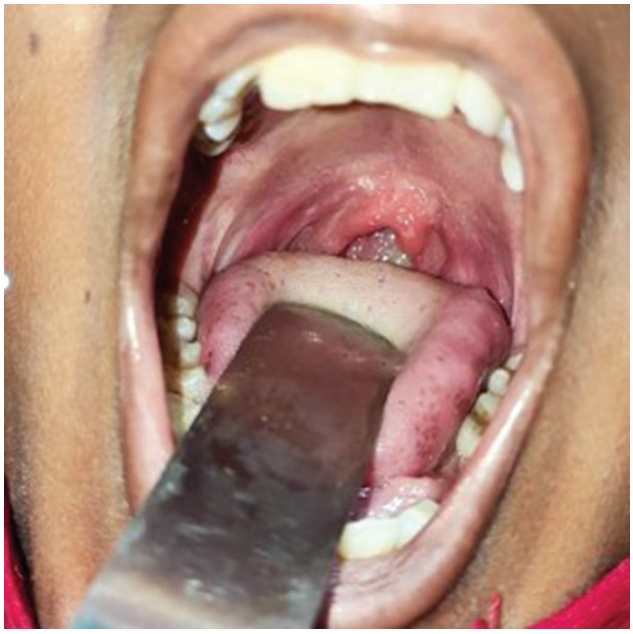

Figure 1. Bifid uvula.

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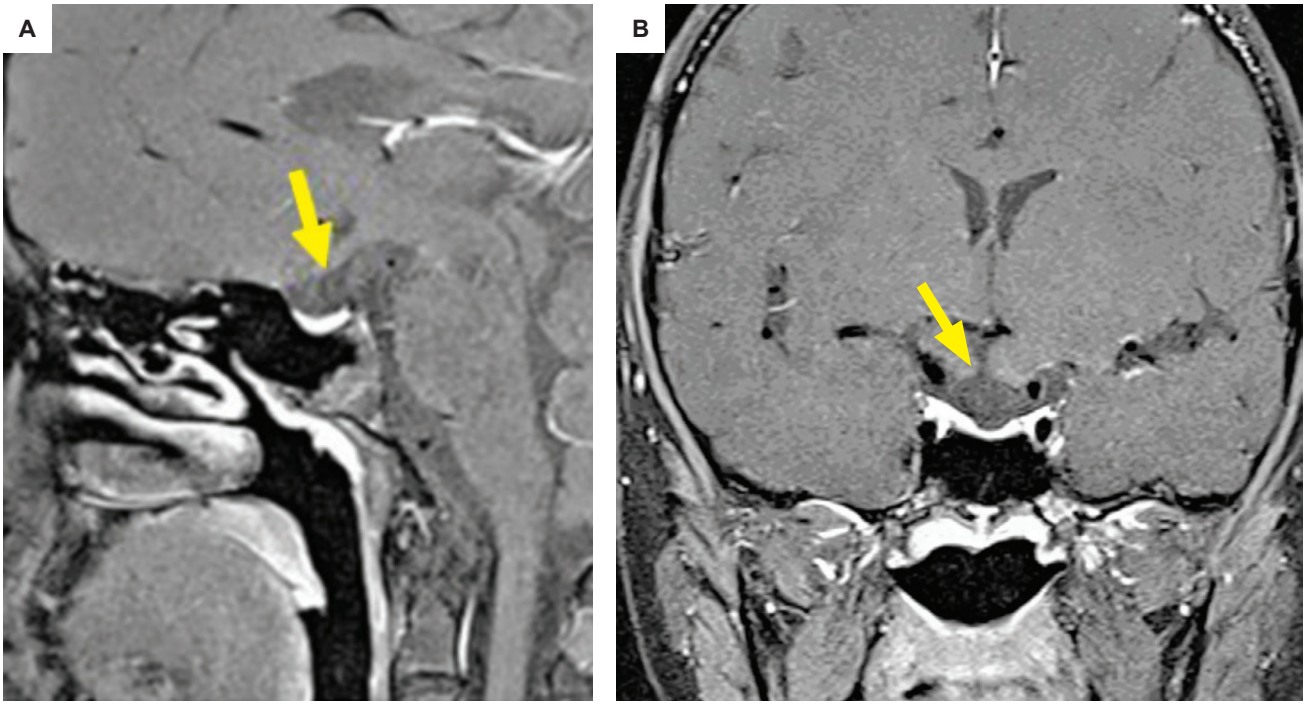

Figure 2. MRI pituitary **(A)** sagittal view, **(B)** coronal view showing hypoplastic anterior pituitary *(yellow arrow)* with eutopic posterior pituitary.

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

Statement of Authorship
Both authors certified fulfilment of ICMJE authorship criteria.

CRediT Author Statement
PS: Conceptualization, Software, Formal analysis, Resources, Data curation. Writing – review and editing, Supervision, Funding acquisition. **AD:** Conceptualization, Methodology, Validation, Investigation, Writing – original draft preparation, Visualization, Project administration.

Author Disclosure
Both authors declared no conflict of interest.

Funding Source
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Prof. Maung has nothing to disclose.

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Dr. Silao receives research grants from the Newborn Screening Reference Center and the National Institutes of Health. She receives fees for lectures from Nestle Philippines. 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 a member of the Human Genome Education Committee. She is a stockholder of the Clinical Genetics and Genomic Counseling Care Services (CGGCCS) Inc.

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Prof. Win has nothing to disclose.

MARITA V.T. REYES | Ethics Adviser
Dr. Reyes receives consulting fees as a Technical Reviewer of study proposals of the Department of Science and Technology-Philippine Council for Health Research Development (DOST-PCHRD). She is the Chair of the Health Technology Assessment Council.

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Dr. Tandoc receives fees as a Consultant Editor of the Scientific Proceedings of the Lung Center of the Philippines. He is a speaker and Treasurer of the Philippine Association of Medical Journal Editors (PAMJE).

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AUTHOR FORM

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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 equip-ment manufacturers, biomedical device manufacturers, or any companies with significant involvement in the field of health care.

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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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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.

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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.

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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.
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   Krause RM. The origin of plagues: Old and new. Science. 1992;257:1073-8. PMID: 1509258. https://doi.org/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. https://doi.org/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. https://doi.org/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. https://doi.org/10/15605/jafes.035.01.10.

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Book

Byrne, DW. Publishing your medical research paper: What they don't teach in medical school. Baltimore: Williams & Wilkins, 1998.

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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

1. Figures or graphs should be identified by Arabic Numeral/s with titles and explanations underneath.
2. The numbers should correspond to the order in which the figures/graphs occur in the text. It is recommended that figures/graphs also be submitted as image files (preferably as .tif, .jpeg, or .png files) of high resolution (at least 300 dpi).
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.
6. Up to a maximum of five (5) figures and graphs are allowed.

Illustrations and Photographs UPDATE

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.
   1.1 There should be minimal processing of digital images submitted with a manuscript for review to the JAFES. A certain degree of image processing (lighting, color, contrast, size, orientation, cropping, placement of identifying markers and labels) is deemed acceptable only if the final image correctly and accurately represents the original information or data. Thus, JAFES requires all unprocessed, unaltered, and raw image files to be submitted with the manuscript to facilitate evaluation and review. These shall serve also as JAFES' records for issues that may arise after publication of the manuscript.
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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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PROCESS

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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4. 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, (c) major manuscript revision and resubmission, or (d) not accepted for publication.
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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.

Case Reports / Case Series
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.

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JAFES encourages submission of special articles that summarize and document the proceedings of endocrinology grand rounds, which includes presentation of medical problems of a particular patient, evaluation and work-up, treatment and clinical course, discussion of key diagnostic and management points, and commentaries by specialty experts. JAFES recognizes the importance of this type of article as an educational tool for physicians and health practitioners. The abstract should be from 50 to 75 words and should not be structured. A manuscript for grand rounds should not exceed 25 typewritten pages (including tables, figures, illustrations and references) or 6000 words.

Brief Communications
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.

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Images may include photographs of clinical cases encountered and documented during practice. They may also include diagnostic images (e.g., photomicrographs of histopathologic diagnosis, radiographs) or special studies performed (e.g., spectral karyotype imaging, fluorescent microscope images, immunostains) that aided in diagnosis. A 250-word text should accompany the images. Submissions to this category should comply with the journal's image integrity guidelines.

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.

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Cover Letter

(Date)

To: **The Editor-in-Chief**
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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.

Attached herewith are the following: the completely accomplished **Author Form with author contribution disclosure** and **author publishing agreement**, in which all the authors certified authorship criteria was satisfactorily met and the specific contributions of the authors are listed and the author copyright is retained granting publishing and distribution rights to the JAFES; the **Author Declaration** that the work is original and is not under simultaneous consideration in other journals and the **ICMJE Disclosure forms** of ALL the authors (*where all conflicts of interest have been declared/there are no conflicts of interest*).

For original articles, we submit a scanned copy of our Ethics Review Approval/registration in trial registries (as appropriate) and the appropriate EQUATOR Network checklist used in writing the manuscript.

For case reports/series, patient consent forms have been secured for the publication of information.

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Furthermore, we respectfully suggest the following **reviewer**(s) for our manuscript.

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1. Zaccardi F et al. *Diabetes Obes Metab.* 2020 Aug 5. Doi:10.1111/dom.14169

COMPOSITION* Diamicron 60 mg MR, modified release tablet containing 60 mg of gliclazide, contains lactose as an excipient. **INDICATION*** Non insulin-dependent diabetes (type 2) in adults when dietary measures, physical exercise and weight loss alone are not sufficient to control blood glucose. **DOSAGE AND ADMINISTRATION*** One half to 2 tablets per day i.e. from 30 to 120 mg taken orally as a single intake at breakfast time, including in elderly patients and those with mild to moderate renal insufficiency with careful patient monitoring. One tablet of Diamicron 60 mg MR is equivalent to 2 tablets of Diamicron 30 mg MR. The breakability of Diamicron 60 mg MR enables flexibility of dosing to be achieved. In patients at risk of hypoglycemia, daily starting dose of 30 mg is recommended. *Combination with other antidiabetics:* Diamicron 60 mg MR can be given in combination with biguanides, alpha glucosidase inhibitors or insulin (under close medical supervision). **CONTRAINDICATIONS*** Hypersensitivity to gliclazide or to any of the excipients, other sulfonylurea or sulphonamides; type 1 diabetes; diabetic pre-coma and coma, diabetic ketoacidosis; severe renal or hepatic insufficiency (in these cases the use of insulin is recommended); treatment with miconazole (see interactions section); lactation (see fertility, pregnancy and lactation section). **WARNINGS *** Hypoglycemia may occur with all sulfonylurea drugs, in cases of accidental overdose, when calorie or glucose intake is deficient, following prolonged or strenuous exercise and in patients with severe hepatic or renal impairment. Hospitalization and glucose administration for several days may be necessary. Patient should be informed of the importance of following dietary advice, of taking regular exercise and of regular monitoring of blood glucose levels. To be prescribed only in patients with regular food intake. Use with caution in patients with G6PD-deficiency. Excipients: contains lactose. **INTERACTION(S)*** Risk of hypoglycemia – *contraindicated:* miconazole; *not recommended:* phenylbutazone; alcohol; *use with caution:* other antidiabetic agents, beta-blockers, fluconazole, ACE inhibitors (captopril, enalapril), H2-receptor antagonists, MAOIs, sulfonamides, clarithromycin, NSAIDs. Risk of hyperglycaemia – *not recommended:* danazol; *use with caution:* chlorpromazine at high doses; glucocorticoids; ritodrine; salbutamol; terbutaline, Saint John's Wort (*hypericum perforatum*) preparations. Risk of dysglycaemia – *use with caution:* fluoroquinolones. Potentiation of anticoagulant therapy (e.g. warfarin), adjustment of the anticoagulant may be necessary. **PREGNANCY*:** Change to insulin before a pregnancy is attempted, or as soon as pregnancy is discovered. **BREASTFEEDING*:** contra-indicated. **FERTILITY* DRIVE & USE MACHINES*** Possible symptoms of hypoglycemia to be taken into account especially at the beginning of the treatment. **UNDESIRABLE EFFECTS*** Hypoglycemia, abdominal pain, nausea, vomiting, dyspepsia, diarrhea, constipation. Rare: changes in haematology generally reversible (anaemia, leucopenia, thrombocytopenia, granulocytopenia). Raised hepatic enzymes levels (AST, ALT, alkaline phosphatase), hepatitis (isolated reports). If cholestatic jaundice: discontinuation of treatment. Transient visual disturbances at start of treatment. More rarely: rash, pruritus, urticaria, angioedema, erythema, maculopapular rashes, bullous reactions such as Stevens-Johnson syndrome and toxic epidermal necrolysis and autoimmune bullous disorders, and exceptionally, drug rash with eosinophilia and systemic symptoms (DRESS). As for other sulfonylureas: observed cases of erythrocytopenia, agranulocytosis, haemolytic anaemia, pancytopenia, allergic vasculitis, hyponatraemia, elevated liver enzymes, impairment of liver function (cholestasis, jaundice) and hepatitis which led to life-threatening liver failure in isolated cases. **OVERDOSE*** Possible severe hypoglycemia requiring urgent IV glucose, immediate hospitalization and monitoring. **PROPERTIES*** Diamicron 60 mg MR is a sulfonylurea reducing blood glucose levels by stimulating insulin secretion from beta cells in the islets of Langerhans, thereby restoring the first peak of insulin secretion and increasing the second phase of insulin secretion in response to a meal or intake of glucose. Independent haemovascular properties. **PRESENTATION*** Box of 30, 90 or 100 tablets of Diamicron 60 mg MR in blister. **LES LABORATOIRES SERVIER**, 50 rue Carnot, 92284 Suresnes cedex France. **www.servier.com . *For complete information, please refer to the Summary of Product Characteristics for your country.**

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