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JAFES

Journal of the
ASEAN Federation of
Endocrine Societies

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EDITORIAL CONTACT INFORMATION: Journal of the ASEAN Federation of Endocrine Societies | Unit 2005, 20th floor, Medical Plaza Ortigas, San Miguel Avenue, Ortigas Center, Pasig City, Philippines 1605 | Editorial Coordinator: Amado O. Tandoc III, MD, FPSP | Telefax: (+632) 8637-3162 | E-mail: JAFES@asia.com; JAFES.editor@gmail.com.

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A Decade of Growth

As the official journal of the ASEAN Federation of Endocrine Societies (AFES) of Indonesia, Malaysia, Philippines, Singapore, Thailand, Vietnam, and Myanmar, JAFES has, since 1982, served as the voice of researchers and clinicians on endocrinology, diabetes, and metabolism in our region. This first issue for 2021 marks the 11th year since we revitalized the journal. With sustained AFES support; steady contributions of our editors and peer reviewers; and renewed interest of investigators and authors, we managed our growing pains and learned critical lessons. Our logo aptly depicts the growing tree as a "symbol of family, a common root spreading out into our rich soil, our cultural heritage that is distinctly Southeast Asian" (Figure 1).[1]

Over the last 10 years, JAFES has grown into an internationally recognized, world-class publication, incorporating best practices on scholarly research. We are 100% open access, making all the science available and accessible to everyone, without asking for subscription or download fees, without levying any article processing charges to submitting authors. JAFES has invested in information-technology driven tools and applications to provide articles that are easily searchable, reader-friendly, and permanent in the online scholarly publishing ecosystem. We put a premium on ethical research, subscribing to international reporting standards. We have improved the communication of findings by converting original articles to visual abstracts that are promoted through social media for both practitioners and lay readers, to enhance patient education.[2] We are proud to say that we are among the pioneers in the region. We look forward to an evaluation of these processes, to help us improve further.

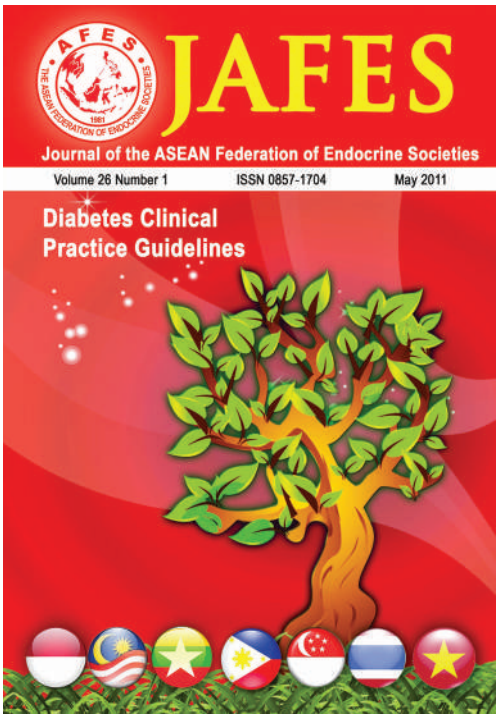

Figure 1. JAFES Vol. 26 No. 1, May 2011 first issue cover designed by Dr. Mia C. Fojas.

JAFES is now indexed in PubMed Central, Scopus™ and Web of Science™ as well as the Western Pacific Region Index Medicus and Directory of Open Access Journals. Indeed, ensuring the visibility of our authors' outputs is a top priority of JAFES.

Relevance and applicability of our publication to our patients in the region is the key goal. We have been highlighting themes of crucial value, starting off with diabetes and the metabolic syndrome, where rates in the region are clearly increasing, occurring at a younger age and with visceral obesity, phenotypes different from our Western counterparts. We have also focused on country practice guidelines, as an initial step towards collaboration within the leaders in the AFES countries. As the COVID-19 pandemic occurred in 2020, AFES editors also initiated a survey on ASEAN needs, with the intent of providing a platform for discussions on these interim reports and practices. We are, in a longer-term view, recognizing the need for understanding endocrine conditions at another level, encouraging molecular and "-omics" research in the region.

Borrowing words from my editorial five years ago, "We feel nostalgic looking back at the work accomplished and the work at hand. With unwavering passion, we reared the journal like our child, full of ambition and hope, celebrating small victories, committing some mistakes in the process, but learning, continually learning, as we go. Each issue, a genuine product of sleepless editorial nights, was like a milestone, from learning to sit to learning to crawl, from crawling to pulling to stand, from standing to finally taking one step, two steps then three."[3] Both as the child and the tree, with commitment and care by AFES, JAFES continues to grow.

Elizabeth Paz-Pacheco
Editor-in-Chief

Postscript:
We are dedicating this issue to Dr. Jose Ma. C. Avila who passed away this year. He was instrumental in providing vital foundations for the revitalized journal, guiding us on the editorial process and teaching us how to manage the journal's operations. Joey was a classmate from medical school, a good friend, a university professor, a leader in Pathology. For this, we will always be grateful.

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ORIGINAL ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

Executive Summary of the 2020 Clinical Practice Guidelines for the Management of Dyslipidemia in the Philippines

Lourdes Ella Gonzalez-Santos,[1] Raymond Oliva,[1] Cecilia Jimeno,[2] Eddieson Gonzales,[3] Maria Margarita Balabagno,[4] Deborah Ona,[1] Jude Erric Cinco,[5] Agnes Baston,[6] Imelda Caole-Ang,[7] Mia Fojas,[8] Ruzenette Felicitas Hernandez,[9] Ma. Cristina Macrohon-Valdez,[10] Maria Theresa Rosqueta,[11] Felix Eduardo Punzalan,[12] Elmer Jasper Llanes[12]

[1]*Division of Hypertension, Department of Medicine, University of the Philippines College of Medicine-Philippine General Hospital*
[2]*Department of Pharmacology and Toxicology, University of the Philippines College of Medicine*
[3]*Department of Internal Medicine, Batangas Medical Center, Philippines*
[4]*Department of Cardiology, Perpetual Help DALTA Medical Center-Las Pinas City, Philippines*
[5]*Department of Cardiology, The Medical City, Pasig City, Philippines*
[6]*Department of Internal Medicine, Emilio Aguinaldo College Medical Center-Cavite, Philippines*
[7]*College of Medicine, Pamantasan ng Lungsod ng Maynila, Philippines*
[8]*Department of Biochemistry and Molecular Biology, University of the Philippines College of Medicine*
[9]*Department of Pediatrics, De La Salle University Medical Center and De La Salle Health Science Institute, College of Medicine, Dasmariñas, Cavite, Philippines*
[10]*Institute of Neurosciences, St. Luke's Medical Center, Global City, Taguig, Philippines*
[11]*Philippine Heart Center*
[12]*Division of Cardiovascular Medicine, Department of Medicine, University of the Philippines College of Medicine-Philippine General Hospital*

Abstract

Dyslipidemia is a cardiovascular risk factor that is increasing in prevalence in the country. The need to treat and manage elevated cholesterol levels, both pharmacologic and non-pharmacologic, is of utmost importance. Different medical societies and groups bonded together to formulate the 2020 Philippine Clinical Practice Guidelines for dyslipidemia. The group raised nine clinical questions that are important in dyslipidemia management. A technical working group analyzed the clinical questions dealing with non-pharmacologic management, primary prevention for both non-diabetic and individuals with diabetes, familial hypercholesterolemia, secondary prevention, adverse events of statins and the use of other lipid parameters as measurement of risk for cardiovascular disease. Randomized controlled trials and meta-analyses were included in the GRADE-PRO analysis to come up with the statements answering the clinical questions. The statements were presented to a panel consisting of government agencies, members of the different medical societies, and private institutions, and the statements were voted upon to come up with the final statements of the 2020 practice guidelines. The 2020 CPG is aimed for the Filipino physician to confidently care for the individual with dyslipidemia and eventually lower his risk for cardiovascular disease.

Key words: guidelines, dyslipidemia, cardiovascular prevention, familial hypercholesterolemia, diabetes mellitus

INTRODUCTION

The 2020 Clinical Practice Guidelines (CPG) for dyslipidemia is a collaboration of different stakeholders in the field of dyslipidemia, particularly the Philippine Heart Association (PHA), the Philippine Lipid and Atherosclerosis Society (PLAS), and the Philippine Society of Endocrinology, Diabetes, and Metabolism (PSEDM). Because of the different issues regarding special populations, the Philippine Society of Nephrology (PSN), Philippine Neurological Association (PNA), and the Philippine Pediatric Society (PPS) became part of this guideline formation. These guidelines are meant to provide physicians with a review of the latest available researches in the field of dyslipidemia to produce recommendations adaptable locally in the Philippines.

This is an update of the 2015 Clinical Practice Guidelines on the Management of Dyslipidemia in the Philippines (2015 CPG).[1] The 2015 CPG covered topics on primary and secondary prevention, non-pharmacologic and pharmacologic treatment of dyslipidemia. When it was released, there were several questions on the management of special populations such as in kidney disease and the pediatric population. There were also issues on the adverse events of statin therapy. There were new clinical data that came out, particularly on ezetimibe, for secondary prevention that were not included in the 2015 CPG.

The objective of the 2020 CPG on Dyslipidemia is to provide evidence-based recommendations to effectively manage individuals with dyslipidemia. These recommendations aim to identify effective and feasible treatment regimens,

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Corresponding author: Raymond V. Oliva, MD
Division of Hypertension, Department of Medicine,
University of the Philippines College of Medicine-Philippine General Hospital
Taft Avenue, Ermita 1000 Manila, Philippines
Tel. No.: +632-85264550
E-mail: rvoliva@up.edu.ph
ORCiD: https://orcid.org/0000-0001-8432-486X

both non-pharmacologic and pharmacologic, in dyslipidemia treatment.

METHODOLOGY

Experts in the fields of dyslipidemia, cardiology, endocrinology, pediatrics, neurology, nephrology and clinical epidemiology assembled to comprise the technical research committee (TRC). They reviewed the recommendations in the 2015 CPG,[1] gathered questions frequently asked in lipid fora conducted in the past five years and discussed challenges in cholesterol management in the Philippines before proposing critical clinical questions to be answered by the 2020 CPG. These questions were presented to the steering committee for comments. Clinical questions were formulated by identifying the specific population, intervention and outcomes for each question. Systematic searches for relevant studies were carried out.

Various clinical outcomes were rated and ranked using the Grades of Recommendation, Assessment, Development and Evaluation (GRADE)[2] categories of importance. The clinical outcomes were rated numerically on a 1-to-9 scale following the GRADE categories, where a score of 7-9 is critical; 4 -6 important; and 1- 3, of limited importance (Table 1). According to GRADE, ranking outcomes by their relative importance can help to focus attention on those outcomes that are considered most important and help to resolve or clarify disagreements.

Table 1. Clinical outcomes used in 2020 Clinical Guidelines

Clinical Outcomes	GRADE Category	Score
Total mortality	Critical	9
Cardiovascular death	Critical	9
Fatal and non-fatal myocardial infarction	Critical	9
Stroke or cerebrovascular disease	Critical	9
Major adverse CV events	Important	7
Coronary revascularization	Important	6

CV, Cardiovascular; GRADE, Grading of Recommendations, Assessment, Development and Evaluations

Cardiovascular events were ranked as CRITICAL with a Score of 7. Coronary revascularization was assigned to be an IMPORTANT outcome with a GRADE PRO Score of 6. Additional important outcomes were added when deemed necessary for the particular clinical scenario (e.g., angina in ACS). Data on these six outcomes were extracted from the retrieved studies.

The TRC members also looked at the most common adverse events affecting individuals on statin treatment. The following outcomes were analyzed using the GRADE-PRO software and were given the following scores (Table 2).

Table 2. Adverse events outcomes used in 2020 Clinical Guidelines

Adverse Outcomes	GRADE Category	Score
Hepatotoxicity	Critical	8
Rhabdomyolysis	Critical	8
Hemorrhagic conversion	Important	6
New onset diabetes	Important	6
Myopathy	Important	6
Intracranial hemorrhage	Important	6
Elevated liver transaminases	Important	6
Risk of dementia	Low	3

GRADE, Grading of Recommendations, Assessment, Development and Evaluations

The TRC searched for all published studies, both local and international, pertaining to the clinical questions, with the use of electronic search engines and manual search. The literature search was conducted using the search engines PubMed, Scopus, Medline, Google Scholar, Cochrane reviews and other medical engines using search words relevant to each clinical question. The cut-off date of the search was February 1, 2020. Unpublished data were also retrieved, whenever possible. To formulate the recommendations, the Working Group used randomized controlled trials (RCTs), meta-analyses, and systematic reviews of studies carried out in individuals with or without established coronary heart disease/CVD and with or without risk factors for coronary heart disease/CVD, and diagnosed with elevated blood cholesterol. Prospective cohorts relevant to the clinical questions are discussed but were not included in the GRADE-PRO analysis.

Standardized tables were used to present the quality of the evidence and key results in a transparent and reproducible fashion. The statements were presented to a panel of experts who voted as to the level of recommendation using the Modified Delphi technique.[3] This technique utilizes consensus strategy that systematically uses literature review, opinion of stakeholders and judgment of experts within a field to reach an agreement. It relies on the collective intelligence of group of members resulting in increased content validity.

THE 2020 CLINICAL PRACTICE GUIDELINES

Clinical Question 1. Lifestyle Modifications

Among individuals with dyslipidemia, regardless of their present condition or risk profile, should lifestyle modification (i.e., reduced fat diet, smoking cessation, regular physical activity) be advised to reduce overall cardiovascular risk?

Statements

- For individuals at any level of cardiovascular risk, a low-fat, low-cholesterol diet, rich in fruits and vegetables, is RECOMMENDED.
- For individuals at any level of cardiovascular risk, cigarette smoking cessation is STRONGLY RECOMMENDED.
- For individuals at any level of cardiovascular risk, e-cigarette smoking/vaping CESSATION IS RECOMMENDED
- For individuals at any level of cardiovascular risk, adequate exercise is RECOMMENDED.

The practice guidelines recommend that lifestyle change play a major role in the management of dyslipidemia. A low-fat, low-cholesterol diet is recommended. We recommend that the Filipino individual with dyslipidemia utilize the *Pinggang Pinoy* that is advocated by the Food Nutrition and Research Institute of the Department of Health. It is a serving plate where half of the portion is of green and leafy vegetables, one-fourth serving of meat and the rest are fiber-rich carbohydrates.

The guidelines also strongly recommend that patients should stop smoking. The use of vaping or e-cigarettes is also not recommended for individuals with dyslipidemia. Exercising at least 150 minutes per week at moderate to high-intensity is also recommended.

PRIMARY PREVENTION

Clinical Question 2.1. Individuals with no prior ASCVD

- Among non-diabetic individuals without ASCVD but with multiple risk factors, should statin therapy be given?

Statement

- For individuals without diabetes aged ≥45 years with LDL-C ≥130 mg/dL AND ≥2 risk factors*, without atherosclerotic cardiovascular disease, statins are RECOMMENDED for the prevention of cardiovascular events.

In the 2015 CPG[1], we identified Risk Factor Counting as the method in identifying the risk of the Filipino individual for cardiovascular disease. We continue to recommend this as the available risk factor scores advocated by the different guidelines (e.g., ASCVD score) are not validated for Filipinos. The following risk factors were identified and if the individual has two (2) or more risk factors, (male sex, postmenopausal women, smoker, hypertension, BMI >25 kg/m^2, family history of premature CHD, proteinuria, and left ventricular hypertrophy) the benefit for statin therapy in primary prevention is fulfilled and statin therapy is indicated.

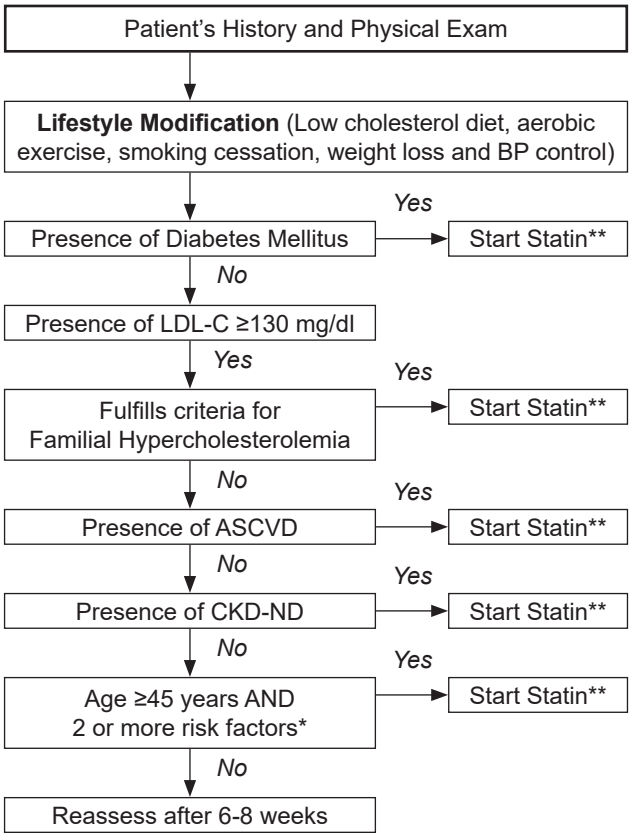


Figure 1. Screening and treatment algorithm for the management of dyslipidemia.

Legend:
* Risk factors: male, smoker, hypertension ≥140/90 mmHg, BMI 25 kg/m^2, family history of premature coronary heart disease, proteinuria, left ventricular hypertrophy and postmenopausal women
** The guideline recommends maximally-tolerated statin therapy to reach recommended target LDL-C levels

Figure 1 is a simplified algorithm that is advocated by the Philippine CPG in the management of dyslipidemia.

The use of statins for individuals with no clinical ASCVD (primary prevention) is recommended for patients aged 45 years and above with 2 or more risk factors with an LDL-C ≥130 mg/dL.

Clinical Question 2.2. Primary Prevention for Individuals with Diabetes Mellitus

- Among individuals with diabetes without ASCVD, should statins be recommended?

Statement

- For individuals with diabetes without evidence of ASCVD, statins are RECOMMENDED for primary prevention of cardiovascular events.

Evidence on the use of statins for primary prevention of cardiovascular outcomes were derived from 8 different clinical trials. The statin dose should be optimized to reach the LDL goal of less than 100 mg/dL for most persons with diabetes for primary prevention. For individuals with diabetes with >1 risk factor (refer to risk factors identified in primary prevention clinical question 2.1), LDL-C goal of less than 70 mg/dL is recommended. An LDL-C of <55 mg/dL should be attained for secondary prevention in individuals with diabetes who are at extremely high risk of having recurrent CV events due the previous occurrence of major cardiovascular events such as myocardial infarction, unstable angina or CVD (stroke).

Clinical Question 3. Familial Hypercholesterolemia

- Among high risk individuals identified to have familial hypercholesterolemia, should statin therapy be initiated?

Statement

- For individuals identified to have familial hypercholesterolemia, statin therapy is STRONGLY RECOMMENDED for the prevention of cardiovascular events.

Familial hypercholesterolemia (FH) is a dominantly inherited gene disorder resulting from gene mutations in the LDL-receptor pathway that cause markedly elevated LDL-C from birth. Untreated FH leads to premature death from coronary artery disease due to accelerated atherosclerosis. Early diagnosis and treatment are vital in prevention of CV events in this high-risk population (Dutch Lipid Network Criteria) (Table 3). The recommendations in this local guideline is to give statin therapy for all identified FH patients for primary prevention. For familial hypercholesterolemia, the recommended statin therapy is the high-intensity dose of statin, with a target LDL-C of less than <70 mg/dl in FH patients without target organ damage, and <55 mg/dl for FH individuals with target organ damage.

Clinical Question 4. Dyslipidemia in the Pediatric Population

- Among pediatric population at risk for premature cardiovascular disease, should screening with fasting lipid profile be recommended?

Table 3. Dutch lipid network criteria on the diagnosis of heterozygous familial hypercholesterolemia[4]

Criteria	Points
Family history	
First-degree relative with known premature* coronary and vascular disease, OR First-degree relative with known LDL-C level above the 95th percentile	1
First-degree relative with tendinous xanthomata and/or arcus cornealis OR Children aged less than 18 years with LDL-C level above the 95th percentile	2
Clinical history	
Patient with premature* coronary artery disease	2
Patient with premature* cerebral or peripheral vascular disease	1
Physical examination	
Tendinous xanthomata	6
Arcus cornealis prior to age 45 years	4
Cholesterol levels in mg/dl (mmol/liter)	
LDL-C ≥330 mg/dL (≥8.5)	8
LDL-C 250 – 329 mg/dL (6.5–8.4)	5
LDL-C 190 – 249 mg/dL (5.0–6.4)	3
LDL-C 155 – 189 mg/dL (4.0–4.9)	1
DNA analysis	
Functional mutation in the LDLR,[b] apo B[c] or PCSK9[d] gene	8
Diagnosis (diagnosis is based on the total number of points obtained)	
Definite familial hypercholesterolemia	>8
Probable familial hypercholesterolemia	6-8
Possible familial hypercholesterolemia	3-5
Unlikely familial hypercholesterolemia	<3

* Premature: ≤55 years in men; <60 years in women; LDL-C, low density lipoprotein cholesterol; FH, familial hypercholesterolemia; LDLR, low density lipoprotein receptor; Apo B, apolipoprotein B; PCSK9, proprotein convertase subtilisin/kexin type 9.

Statement

- Among pediatric population (≤19 years old) at risk for development of atherosclerosis and premature cardiovascular disease, screening with a fasting lipid profile is RECOMMENDED.

The screening for dyslipidemia in childhood is made on the basis of early identification and control of pediatric dyslipidemia that will reduce the risk and severity of cardiovascular disease (CVD) in adulthood (Table 4). There has been no solid evidence so far with regards the screening recommendation for dyslipidemia among the pediatric age group. In this CPG, the screening guidelines that have been suggested are the following: Selective screening for those at risk. Universal screening to identify those with risk factors for familial hypercholesterolemia at 9-11 years old and 17-21 years old.

Table 4. Risk factors for cardiovascular disease in the pediatric population[5,6]

Traditional Risk Factors	Other conditions
Dyslipidemia	• Familial hypercholesterolemia
Obesity	• Chronic kidney disease
Diabetes mellitus (Type 1 or 2)	• Kawasaki disease
Hypertension	• Childhood cancer
Family history of premature CVD	• Transplant vasculopathy
Smoke exposure	• Certain congenital heart disease (e.g., coarctation of the aorta, aortic stenosis) • Cardiomyopathy • Chronic inflammatory disorders • HIV infection • Adolescent depressive and bipolar disorders

CVD, cardiovascular disease; HIV, human immunodeficiency virus

Clinical Question 5. Individuals with Chronic Kidney Disease

- Among individuals with chronic kidney disease who are not on dialysis, should statins be given to reduce CV risk?

Statement

- Among individuals with chronic kidney disease who are not on dialysis, statins are RECOMMENDED for the prevention of cardiovascular events.

Dyslipidemia is common but not universal in CKD. Kidney dysfunction leads to a profound dysregulation of lipoprotein metabolism, resulting in multiple lipoprotein abnormalities. The recommendation in this local guideline is to give statins for individuals with CKD not on dialysis. For individuals who are on renal replacement therapy and post-transplant, this local guideline recommends referring patients to nephrologists for lipid management.

SECONDARY PREVENTION

Clinical Question 6. Individuals with Acute Coronary Syndrome

- Among individuals with acute coronary syndrome (ACS), should statins be given?

Statements

- For individuals with ACS, early high-intensity statin that is maximally-tolerated is RECOMMENDED and should not be discontinued.
- Statins should be given to ACS patients immediately

Timing of therapy is critical among patients with acute coronary syndrome. Early intervention is advocated to optimize recovery and minimize complications. The adage "time is muscle," is based on the principle of the necessity for immediate action during the golden period in which myocardial ischemic damage is still potentially reversible or myocyte necrosis can still be contained and much of the myocardium in the ischemic penumbra can still be salvaged.

Evidence on the appropriate statin intensity for secondary prevention in individuals with ASCVD were obtained from four (4) trials[7-10] that compared varying statin regimens. High-intensity statins reduce LDL-C by >50% compared to low-intensity statins which reduce LDL-C by less than 30% (Table 5).

Table 5. Statin treatment intensity

Treatment intensity	% LDL-C reduction	Drug regimen
Low intensity	<30 %	Fluvastatin 20 - 40 mg Pravastatin 10 - 20 mg Simvastatin 10 mg
Moderate intensity	30% - 50%	Atorvastatin 10 - 20 mg Fluvastatin 80 mg Rosuvastatin 5 - 10 mg Simvastatin 20 - 40 mg Pravastatin 40 - 80 mg Pitavastatin 2 - 4 mg
High intensity	>50%	Atorvastatin 40 - 80 mg Rosuvastatin 20 - 40 mg

LDL-C, low density lipoprotein cholesterol

NON-STATIN THERAPY

Clinical Question 7.1. Use of Ezetimibe

- Among individuals with ASCVD, should ezetimibe be given on top of statin therapy?

Statement

- For individuals with documented ACS, and target LDL-C has not been reached despite maximally-tolerated high-intensity statin therapy, ezetimibe may be added on top of statin therapy to get to goal LDL-C.

Ezetimibe is an important adjunct medication in lowering LDL-C in the body. This guideline recommends that ezetimibe be given to patients with documented ACS on maximally tolerated statin therapy not at goal LDL-C levels.

Clinical Question 7.2. Use of Fibrates

- Among individuals with ASCVD, should fibrates be given on top of statin therapy once LDL-C goal is achieved?

Statements

- Among individuals without diabetes not at goal LDL-C, routinely adding fibrates on top of statin therapy is NOT RECOMMENDED for primary or secondary prevention of cardiovascular disease.
- Among individuals with diabetes, routinely adding fibrates on top of statin therapy is NOT RECOMMENDED for primary or secondary prevention of cardiovascular disease.
- However, adding fibrates to statins may be considered among MEN with controlled diabetes, low HDL-C (<35 mg/dl) and persistently **high triglycerides (>200 mg/dl)** for prevention of CV disease.

Clinical Question 7.3. Use of Omega Fatty Acids

- Among individuals with ASCVD, should omega fatty acids be given on top of statin therapy once LDL-C goal is achieved?

Statements

- Among individuals with ASCVD, omega fatty acids (EPA+DHA) given on top of statin therapy is NOT RECOMMENDED.
- Among individuals with ASCVD on statin therapy at goal LDL-C, but with persistently high triglyceride levels of 150-499 mg/dl, omega fatty acids (pure EPA) MAY be given.

FOUR PATIENT GROUPS

The 2020 CPG recommends that individuals be divided into four patient groups (Table 6). The cholesterol targets are based on clinical data and expert opinions of the voting panel.

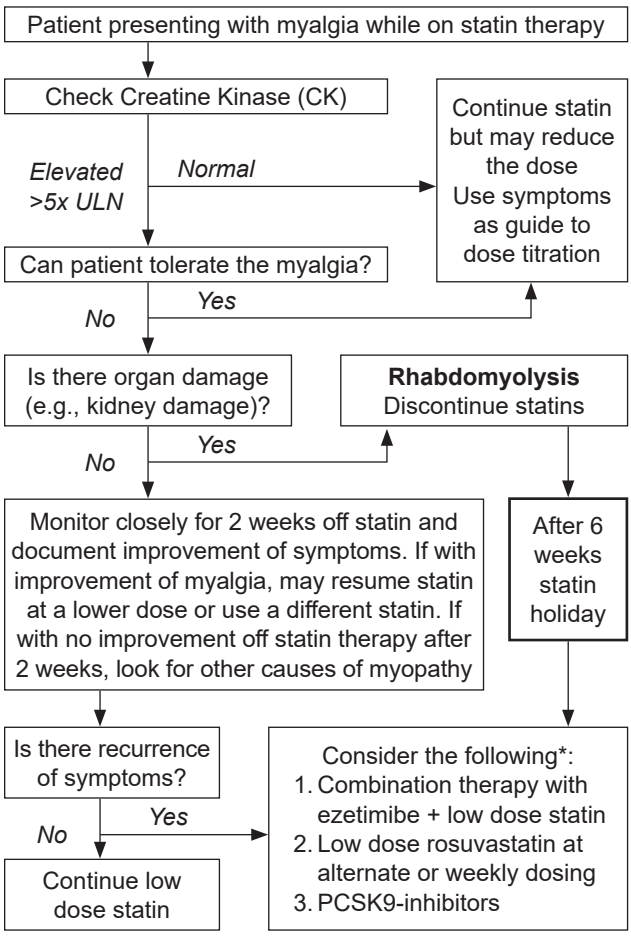
STATIN ADVERSE EVENTS

Clinical Question 8. Use of Statin Therapy

- Among individuals taking statin therapy, what is the risk of developing adverse effects?
  a. Statin-associated Muscle Symptoms
  b. New-onset Diabetes
  c. Dementia / cognitive dysfunction / intracerebral hemorrhage

Statements

- Treatment with statins is associated with a low risk of developing statin-associated muscle symptoms (SAMS), but the benefits of cardiovascular risk reduction outweigh the risk (Figure 2).
- Treatment with statins is associated with an increased risk of new-onset diabetes mellitus, but the benefits



* If symptoms recur after multiple statin use at multiple dosing, may use non-statin therapy (fibrates or ezetimibe)

Figure 2. Algorithm for Statin-induced Myopathy.

Table 6. Cholesterol targets for different patient groups

Patient Groups	LDL-C Target	HDL-C Target	Triglyceride Target
Individuals with no clinical ASCVD	<130 mg/dL	>40 mg/dl in males / >50 mg/dl in females	<150 mg/dl
Individuals with DM	<100 mg/dL	>40 mg/dl in males / >50 mg/dl in females	<150 mg/dl
With ≥1 risk factors / target organ damage	<70 mg/dL		
With ASCVD	<55 mg/dL		
Individuals with clinical ASCVD	<55 mg/dL	>40 mg/dl in males / >50 mg/dl in females	<150 mg/dl
FH without ASCVD or without major risk factor / target organ damage	<70 mg/dL	>40 mg/dl in males / >50 mg/dl in females	<150 mg/dl
FH with ASCVD or with ≥1 risk factors / target organ damage	<55 mg/dL		

ASCVD, atherosclerotic cardiovascular disease; DM, diabetes mellitus; FH, familial hypercholesterolemia

of statin treatment for cardiovascular risk reduction outweigh the risk.
- Treatment with statins is not associated with the development of dementia and cognitive dysfunction
- Treatment with statins is not associated with an increased risk of intracerebral hemorrhage

ADDITIONAL TARGETS TO REDUCE CARDIOVASCULAR RISK

Clinical Question 9.1. Use of non-HDL-C

- Among individuals on statin therapy who have achieved their LDL-C goal, should non-high density lipoprotein cholesterol (non-HDL-C) be used as additional target to reduce CV events?

Statement

- Among individuals on statin therapy who have achieved their LDL-C goal, an elevated computed non-HDL-C may be used as an additional therapeutic target to further reduce CV events.

Non-HDL-C is the difference between the total cholesterol levels and HDL-C and quantifies all atherogenic lipoprotein particles. Target non-HDL in various guidelines set it as 30 mg/dL above target LDL-C. In patients with atherogenic dyslipidemia such as those with metabolic syndrome, type 2 diabetes mellitus and obesity, non-HDL-C determination is recommended as an additional tool providing a better estimate of risk beyond LDL-C. The non-fasting HDL computation has prognostic value in clinical trials as a therapeutic target.

Clinical Question 9.2. Use of Apolipoprotein B-100

- Among individuals on statin therapy who have achieved their LDL-C goal, should apolipoprotein B-100 be used as additional target to reduce CV events?

Statement

- Among individuals on statin therapy who have achieved their LDL-C goal, an elevated apolipoprotein B-100 may be used as an additional therapeutic target to further reduce CV event.

Limitations of the Guidelines

Several limitations were encountered during the process of creating the 2020 CPG. The evidence obtained from the trials only involved randomized controlled trials and some meta-analyses. Observational studies were only used as references. This approach, however, resulted in a comprehensive set of evidence-based clinical recommendations. The clinical trials did not include Filipino patients, thus analysis and grading of evidence were downgraded. Thus, we can only RECOMMEND the statements in this guideline. We hope in the future that more clinical trials be made with Filipino patients as subjects.

CONCLUSIONS

The clinical statements were made by the TRC and the recommendations revolve around the holistic management of dyslipidemia. Lifestyle modification should be recommended to all patients regardless of their CVD risk. Dosing of statin therapies should be based on individual risk factors. We recommend a lower LDL-C target, particularly for secondary prevention. The simplified algorithm was provided to serve as a quick reference in the management of dyslipidemia for clinicians.

The 2020 CPG is designed to be a guide for clinicians in managing dyslipidemia for the Filipino patient. This, however, should not replace sound clinical judgment by doctors and the ultimate decision for treatment should involve both clinician and the patient.

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Steering Committee:

Adriel Guerrero (Philippine Lipid and Atherosclerosis Society)
Gilbert Villela (Philippine Heart Association)
Jeremy Jones Robles (Philippine Society of Endocrinology, Diabetes and Metabolism) Maaliddin Biruar (Philippine Society of Nephrology)
Roberto Mirasol (Philippine Lipid and Atherosclerosis Society)
Romeo Esagunde (Philippine Neurological Association)

Expert Panel

Leilani Asis (Philippine Society of Hypertension)
Federick Cheng (Philippine Lipid and Atherosclerosis Society)
Michael Villa (Philippine Society of Endocrinology, Diabetes and Metabolism)
Arlene Afaga (Philippine Society of Nephrology)
Encarnita Limpin (Philippine College of Physicians)
Nemencio Nicodemus, Jr. (Philippine College of Physicians)
Florence Santos (Philippine Medical Association)
Jennifer Manzano (Philippine Neurological Association)
Ma. Bernadette Azcueta (Philippine Pediatric Society)
Jane Eflyn Bunyi (Philippine Academy of Family Physicians)
Emma Gaspar Trinidad (Philippine Heart Association)
Mildred Udarbe (Food and Nutrition Research Institute)
Romeo Merino (Manila Doctors Hospital)
Mark Anthony Cepeda (Philippine Health Insurance) [Non-voting]

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

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ORIGINAL ARTICLE

Cardiometabolic Risk Factors leading to Diabetes Mellitus among the Young (YOD) from the 8th Philippine National Nutrition Survey

Angelique Bea Uy[1] and Cecilia Jimeno[2]

[1]*Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of the Philippines College of Medicine-Philippine General Hospital*
[2]*Department of Pharmacology and Toxicology, University of the Philippines College of Medicine*

Abstract

Objectives. This study looked into the prevalence of diabetes mellitus (DM) and risks for cardiovascular and metabolic diseases among young adults with diabetes (age 20-44 years old, YOD) and late-onset DM (≥45 years old, LOD) in Filipinos.

Methodology. Weighted data from 546,580 adults with DM from the 8th Philippine National Nutrition and Health Survey (NNHeS) were utilized. Differences in sociodemographic, anthropometric, clinical profiles and metabolic risks were compared between YOD and LOD.

Results. The aggregated prevalence of DM is 5.43% (95%CI, 5.10–5.79), YOD were 2.64% (95% CI, 2.32–3.00) and LOD 9.85% (95%CI, 9.18–10.56). Mean age of YOD was 37,6 years, LOD 59,9 years. The YOD were mostly males (56%), with higher BMI (26.24 kg/m^2 vs 25 kg/m^2, p=0.002), lower mean SBP (122.41±19.17 mmHg vs 135.45±22.47 mmHg, p<0.001), more daily smokers (23% vs 14%), and alcoholic beverage drinkers (39% vs 31%). Physical activity was similar between groups (44% vs 51%, p=0.078). However, average total caloric intake (1776.78±758.38 kcal vs 1596.88±639.16 kcal, p=0.023) and carbohydrate intake (306.13±142.16 grams vs 270.53±104.74 g, p=0.014) were higher in YOD. Dietary carbohydrate proportions were higher than recommended (69% vs 68%) for both groups. Young Filipinos had higher risk to develop diabetes when they are obese II (22% vs 12%), current drinker (56% vs 37%), and current smoker (28% vs 18%). Eighty percent of YOD and LOD had metabolic syndrome (MetS). With every unit increase in age and fat intake, the odds of having MetS were raised by 5.4% (95%CI 1%–10%, p=0.029) and 1.6% (95%CI 0.04%-3%, p=0.044), respectively.

Conclusion. Early-onset diabetes mellitus appears to be driven by obesity, MetS and social behaviors. Modifiable risk factors can be improved early to decrease hazards to develop cardiometabolic complications.

Key words: young-onset diabetes mellitus, Filipinos, metabolic syndrome, cardiovascular disease

INTRODUCTION

The growing burden of diabetes among adult populations worldwide cannot be overemphasized. In 2019, it was estimated that 463 million people worldwide have diabetes and this number is projected to reach 578 million by 2030, and 700 million by 2045.[1] This alarming increase in the number of people with diabetes does not only threaten individuals and their families, but has implications on economic and social outcomes in nations and the global population as a whole. The Philippines' data on diabetes mirrors this alarming rise worldwide with diabetes among the top causes of morbidity and mortality in the last two decades.[2]

One of the major aspects on addressing the global epidemic of diabetes is generating information on the pattern and burden of disease among different populations and age groups. In the recent decade, there is a particular concern and emphasis on young adults aged 18-44 with diabetes (YOD) for two compelling reasons: rising prevalence of diabetes in young adult age groups, and accompanying cardiometabolic risk factors early in life.[3,4] In the Philippines, there are no available data yet as to the prevalence of early-onset diabetes. However, as lifestyle and diet of Filipinos shift towards the demands of urbanization and globalization, we expect an increasing trend of emerging health problems brought about by the consumption of foods high in fat, sugar, and salt coupled with unhealthy lifestyle and stressful environments.

The Filipino YOD has not yet been fully characterized using nationally representative local data. This study seeks to fill this gap in literature by using data from the 8th Philippine National Nutrition and Health Survey (NNHeS)[5] which covered 17 regions and 80 provinces of the Philippines. The

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Corresponding author: Angelique Bea C. Uy, MD
Division of Endocrinology, Diabetes and Metabolism
Department of Medicine, University of the Philippines
College of Medicine-Philippine General Hospital
Taft Avenue, Ermita 1000 Manila, Philippines
Tel. No.: +632-55484000
E-mail: acuy2@up.edu.ph
ORCiD: https://orcid.org/0000-0002-9539-8239

objective of this study was to determine the prevalence of cardiovascular and metabolic diseases among the Filipino young adults with diabetes. Specifically, it aimed to determine the demographics and the clinical, behavioral and biochemical cardiometabolic risk factors among young adults aged 20–44 years and compare these with older adults aged 45 years and older with diabetes. This helps us understand the extent to which the risk factors and disease prevalence may differ among the young adult population with diabetes compared to older cohort.

METHODOLOGY

Design

This was a cross-sectional analytic study with data derived from the results of the NNHeS which is available from the public use files (PUF) of the Food and Nutrition Research Institute (FNRI) at the website, *http://enutrition.fnri.dost.gov.ph/site/puf-dataset.php/*. This survey had achieved sample size of 1104 respondents, with weighted count of 546,580.

Description of the Data Source

The 8th Philippine National Nutrition Clinical and Health Survey is a cross-sectional study approved by the FNRI Ethics Review Committee on January 22, 2013. It included a subsample of the Family Income and Expenditure Survey of the National Statistics Office. NNHeS utilized a stratified multi-stage sampling design covering the country's regions and provinces, except for Batanes. The primary sampling units were barangays, from which various enumeration areas were randomly chosen. From these areas, different households were sampled. In all, a total of 2,636 households from 17 regions and 80 provinces were covered between August 2013 and January 2014.

The study used the four-pronged approach of anthropometric, biochemical, clinical and dietary/food consumption assessments. Anthropometric measurements included height, weight, waist and hip circumference. Biochemical examinations were laboratory tests for lipid profile including total cholesterol, HDL-c, LDL-c and triglycerides, and fasting blood sugar. In the 8th NNHeS only one determination of FBS was done. Clinical evaluation included blood pressure monitoring. All forms were checked and rechecked during encoding using a CSPro program version 2.4. Data cleaning, checking for consistency and data processing were done by region. Weights were assigned and attached to the cleaned data so that the distributions in the households would reflect their actual distributions in the population as a whole.

Population

Filipino adults with diabetes who participated in the 2013 NNHeS.

Outcomes

1. Demographic variables – included age, sex, educational attainment, and socioeconomic status.
2. Cardiometabolic profile – This included measures of adiposity such as Body Mass Index (BMI), waist-to-hip ratio and waist circumference–to–height ratio (WHtR). Variables which were analyzed include:
    a. Serum total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), total cholesterol: HDL ratio
    b. Fasting Blood Sugar (FBS)
    c. Blood pressure (BP)
    d. Metabolic Syndrome (MetS)
    e. Smoking Status
    f. Alcoholic beverage intake
    g. Healthy eating habits/ Dietary profile
    h. Leisure-time physical activity (PA)

Operational definitions

1. Young-onset diabetes (YOD) or younger adults with diabetes are those aged 18-44 years old with diabetes. In the 8th NNHeS, FBS measurement started at 20 years and above.
2. UNITE for Diabetes Philippine Clinical Practice Guidelines (CPG) adapted from the American Diabetes Association (ADA) criteria for diabetes and dysglycemia are as follows:
    a. Normal FBS is <100 mg/dL
    b. Impaired fasting glucose is FBS 100-125 mg/dL, and
    c. Diabetes is FBS ≥126 mg/dL
3. Asia-Pacific Classification of BMI was adopted for this study:
    a. Underweight: <18.5 kg/m^2
    b. Normal weight: 18.5-22.9 kg/m^2
    c. Overweight: 23-24.9 kg/m^2
    d. Obese I: 25-29.9 kg/m^2
    e. Obese II: ≥30 kg/m^2
4. WHO-Asia Pacific Classification of waist circumference:
    a. Males: normal (<90 cm), borderline (90-101 cm), and high (>101)
    b. Females: normal (<80 cm), borderline (80-87 cm), and high (>87 cm)
5. Other Obesity indices:
    a. Waist–hip ratio values of greater than 0.90 and 0.80 for men and women, respectively, OR
    b. Waist to Height Ratio (WHtR) of ≥0.5
6. Blood pressure categories in this study are based on the American College of Cardiology/American Heart Association (ACC/AHA) 2017 guidelines on Hypertension
    a. Normal (systolic BP <120 and diastolic BP <80 mmHg)
    b. Elevated (systolic BP 120-129 and diastolic <80 mmHg)
    c. Stage 1 hypertension (systolic BP 130-139 or diastolic 80-89 mmHg)
    d. Stage 2 hypertension (systolic BP ≥140 or diastolic ≥90 mmHg)
7. The 2001 National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) guidelines on serum lipid level categories were used in this study as follows:
    a. Total cholesterol (in mg/dL) desirable (<200), borderline high (200-239), high (>240);
    b. LDL-c (in mg/dL) optimal (<100), near optimal/ above optimal (100-129), borderline high (130-159), high (160-189), very high (>190);
    c. HDL-c (in mg/dL) low (<40), borderline (50- 59), desirable (>60);
    d. Triglyceride (in mg/dL) desirable (<150), borderline (150-199), high (200-399), very high (<400).

8. Alcohol consumption was classified according to these WHO categories (2014):
   a. Lifetime abstainers are people who have never consumed alcohol
   b. Former drinkers are people who have previously consumed alcohol but have not done so in the previous 12-month period, and
   c. Current drinkers are people who were currently consuming alcohol during the survey period.
   d. Binge drinking status for males is defined as drinking five or more standard drinks in a row, while for females it is drinking four or more standard drinks in a row.
   e. Standard drink is equal to 14.0 grams (0.6 ounces) of pure alcohol, with moderate alcohol consumption defined as having up to 1 drink per day for women and up to 2 drinks per day for men
9. Cigarette smoking status was categorized according to the WHO STEPS Surveillance Manual:
   a. Current smokers are those who smoke during the time of survey either on a daily basis (at least 1 cigarette a day), or on a regular/occasional smoking, or those who do not smoke daily but who smoke at least weekly, or those who smoke less often than weekly,
   b. Former smokers are those who have ever smoked in the past year prior to the survey whether in a daily basis or an aggregate lifetime consumption of at least 100 cigarettes but not daily; and
   c. Never smokers are those individuals who have never smoked at all.
10. A person not meeting any of the following criteria is considered physically inactive or insufficiently physically active and therefore at risk for chronic disease based on the WHO STEPS Surveillance Manual:
   a. 3 or more days of vigorous-intensity activity of at least 20 minutes per day or
   b. 5 or more days of moderate intensity activity or walking of at least 30 minutes per day
11. Unhealthy diet is the failure to meet the WHO recommended intake of 400 g of fruits and vegetables per day based on the 24-hour food recall.
12. Metabolic Syndrome defined by the NCEP-ATP III as fulfilling at least 3 out of 5 of the following criteria:
   a. Waist circumference for males: ≥90 cm, females: ≥80 cm;
   b. Triglycerides ≥150 mg/dl;
   c. HDL cholesterol for males: <40 mg/dl, females <50 mg/dl;
   d. Fasting blood sugar ≥100 mg/dl;
   e. Blood pressure: ≥130 mmHg systolic or ≥80 mmHg on antihypertensive drug treatment in a patient with hypertension

Statistical Methods

The study utilized the 8th NNHeS data requested from the FNRI. A single sampling using the dataset weights from the socio-demographic profile was implemented to generate the results. Descriptive statistics were used to summarize the general and clinical characteristics of the participants. Frequency and proportion were used for nominal variables, median and range for ordinal variables, and mean and standard deviation for interval/ratio variables.

Adjusted Wald test was used to determine the difference of mean between the two age groups. Pearson's Chi-square test and logistic regression were used to determine differences in the frequency and risk between groups.

All valid data were included in the analysis. Missing variables were neither replaced nor estimated. Null hypothesis was rejected at 0.05 α-level of significance. STATA 15.0 was used for data analysis.

Ethical Issues

The study was conducted in compliance with the ethical principles set forth in the Declaration of Helsinki and the (Philippine) National Ethical Guidelines for Health and Health-Related Research of 2017. The study protocol and subsequent amendments underwent review and approval by the UP Manila Research Ethics Board (UPM REB) prior to study initiation.

RESULTS

A total of 100,021 adults aged 20 years or older were identified from the NNHeS dataset, of whom 18,484 individuals had fasting blood sugar levels tested. Elevated blood sugar was found among 1,104 of the 18,484 tested. The estimated weighted prevalence of DM in the adult population is 5.43% (95% CI, 5.10–5.79). Disaggregated, DM prevalence in the young adult (20–44 years) and ≥45 years age brackets were 2.64% (95% CI, 2.32–3.00) and 9.85% (95% CI, 9.18–10.56), respectively.

Characteristics of Young Adults with Diabetes

Mean, SD ages of the young adult and older adult age groups were 37, 6 years and 59, 9 years, respectively. Males comprised a greater proportion of young adults (56%) compared to older adults (45%). Most of YOD were high school graduates, while LOD finished elementary education. Majority in both groups were married and employed. Those belonging to the two highest socioeconomic quintiles (Q4-Q5) comprised 51% and 57% of young and older adults, respectively (Table 1).

The young adults had significantly greater weight (66.36±14.24 kg vs. 61.35±11.81 kg, $p<0.001$), higher BMI (26.24 kg/m^2 vs 25 kg/m^2, $p=0.002$), and had more obese II proportions than older adults (22.38% vs. 12.14%, $p=0.002$). Distributions of waist ($p>0.999$) and hip ($p=0.273$) circumferences among the young and older adults were not found to be significantly different. While the mean SBP of younger adults was significantly lower compared to that of older adults (122.41±19.17 mmHg vs 135.45±22.47 mmHg, $p<0.001$), their diastolic BP was not statistically significantly different ($p=0.188$). A greater proportion among the YOD, as compared to LOD, were presently daily smokers (23% vs 14%). Although significantly more of the older adults, in comparison with the younger group, had never consumed alcoholic beverage (48% vs 31%), there was no statistically significant difference in proportions of binge drinkers between the two groups (11% vs 16%, respectively). Physical activity was likewise determined to be not significantly different between the two groups ($p=0.078$). However, despite having a greater proportion who were physically active among the YOD compared to LOD (55.55% versus 48.38%), there are more obese persons among the young (56% vs 49%) (Table 2).

Table 1. Socio-demographic profile of NNHeS adult respondents with diabetes ($n_{weighted}$=546580)

	All (n_w=546580)	Young Adults (n_w=162675)	Older Adults (n_w=383905)	*p*
	Frequency (%); Mean ± SD			
Age, years	52.18 ± 13.06	36.79 ± 6.40	58.71 ± 9.08	-
Sex				.004*
Male	262715 (48.07)	91027 (55.96)	171688 (44.72)	
Female	283864 (51.93)	71648 (44.04)	212217 (55.28)	
Civil status				<.001*
Single	67889 (12.42)	39844 (24.49)	28046 (7.31)	
Married	371373 (67.94)	106697 (65.59)	264676 (68.94)	
Live-in	26181 (4.79)	12459 (7.66)	13722 (3.57)	
Widowed	68070 (12.45)	1285 (0.79)	66784 (17.4)	
Separated	13067 (2.39)	2390 (1.47)	10677 (2.78)	
Highest educational attainment				<.001*
No grade completed	11430 (2.09)	1688 (1.04)	9742 (2.54)	
Nursery/Kinder/Preparatory	571 (0.1)	0	571 (0.15)	
Some Elementary	76178 (13.94)	11790 (7.25)	64387 (16.77)	
Elementary Graduate	97800 (17.89)	19040 (11.7)	78760 (20.52)	
Some High School	65283 (11.54)	22762 (13.99)	42521 (11.07)	
High School Graduate	121143 (22.16)	46601 (28.65)	74543 (19.42)	
Some Voc/Tech	5566 (1.02)	2084 (1.28)	3482 (0.91)	
Graduate (Voc/Tech)	26877 (4.92)	9050 (5.56)	17826 (4.64)	
Some College	52343 (9.58)	18702 (11.5)	33641 (8.76)	
College graduate	85168 (15.58)	30148 (18.53)	55020 (14.33)	
Master's graduate	3158 (0.58)	406 (0.25)	2752 (0.72)	
PhD graduate	347 (0.06)	0	347 (0.09)	
Others	403 (0.07)	403 (0.25)	0	
Missing	313 (0.06)	0	313 (0.08)	
Occupational status				<.001*
No occupation	97921 (17.92)	28046 (17.24)	69875 (18.2)	
Housekeeper	97726 (17.88)	22883 (14.07)	74843 (19.5)	
Student	4012 (0.73)	3385 (2.08)	627 (0.16)	
Pensioner	34339 (6.28)	0	34339 (8.94)	
With job or business	311430 (56.98)	108361 (66.61)	203069 (52.9)	
With job or business and Student	1152 (0.21)	0	1152 (0.3)	
Wealth quintile				.155*
Poorest	62858 (11.78)	24791 (15.51)	38067 (10.18)	
Poor	83375 (15.62)	23003 (14.39)	60372 (16.15)	
Middle	93619 (17.54)	31221 (19.53)	62398 (16.69)	
Rich	129118 (24.2)	36838 (23.05)	92280 (24.69)	
Richest	164659 (30.86)	43993 (27.52)	120666 (32.28)	

Statistical tests used: * - Pearson's chi-square test

In terms of patterns of dietary intake (Table 3), there was notably greater consumption of cereals (p=0.002) and rice (p=0.028) among young adults as compared to older ones, but the latter consumed more starchy roots and tubers (p=0.008), as well as milk and its derivatives (p=0.004). On the other hand, significant differences in intake or consumption of corn, sugars and syrups, dried beans and nuts, vegetables, fruits, fats and oils, fish, meats, poultry, eggs, beverages, condiments, vitamins, minerals, total protein, and total fat, were not detected. Though the total amounts of food consumed were comparable (p=0.690), the average total caloric (1776.78±758.38 kcal vs 1596.88±639.16 kcal, p=0.023) and carbohydrate (306.13±142.16 grams vs 270.53±104.74 grams, p=0.014) intakes were higher in the young adult group than in the older adult group. Carbohydrate intake for both groups comprised 69% and 68%, respectively. Levels of UIE (139.3±131.7 μg/dl vs 105.96±108.65 μg/dl) and hemoglobin (14.5±1.6 g/dL vs 13.9±1.5 g/dL) were significantly greater in the young adult bracket than in the older age group. In contrast, levels of vitamin A were comparable between the two.

Cardiometabolic Risk Factors

Metabolic syndrome was found in 83.76% (81.28-85.97) of the overall population. MetS in the YOD comprised 81.13% (75.59-85.66), and in LOD 84.88% (82.13-87.27). Among the YOD, the diagnostic criteria that were met are as follows: waist circumference more than cutoff among 55%; hypertriglyceridemia was found in 66%; low HDL-C in 84%; elevated fasting glucose 100%; and elevated BP 38% (Table 4).

Lipid profile for both the young and adult age groups had high TC (63% vs 70%, p=0.051), high LDL (94% vs 96%, p=0.119), high Tg (>150 mg/dl) (65% vs 65%, p=0.305), and low HDL (84% vs 82%, p=0.667). Mean values for TC is 215 mg/dl vs 228 mg/dl (p=0.002), LDL 136 vs 150 mg/dl (p<0.001), Tg 228 mg/dl vs 213 mg/dl (p=0.174); and HDL 34 mg/dl vs 35 mg/dl (p=0.316), among the young and older adults with diabetes, respectively. The LOD had significantly higher TC and LDL.

In addition, the young adult group had significantly higher proportions who were in the obese II category (22% vs 12%) using BMI, current drinkers (56% vs 37%), and

Table 2. Anthropometric and Clinical profile of NNHeS adult respondents with diabetes

	All (n_w=546580)	Young Adults (n_w=162675)	Older Adults (n_w=383905)	*p*
		Mean ± SD		
Weight, kg	[n_w=534262]	[n_w=160675]	[n_w=373587]	
	62.86 ± 12.79	66.36 ± 14.24	61.35 ± 11.81	<.001*
Height, cm	[n_w=532651]	[n_w=160675]	[n_w=371976]	
	157.32 ± 8.44	158.95 ± 8.61	156.61 ± 8.27	.001*
BMI, kg/m^2	[n_w=532210]	[n_w=160675]	[n_w=371535]	
	25.38 ± 4.57	26.24 ± 5.05	25 ± 4.29	.002*
BMI classification	[n_w=532210]	[n_w=160675]	[n_w=371535]	.010†
Underweight (<18.5)	28848 (5.42)	7982 (4.97)	20866 (5.62)	
Normal (<23)	143652 (26.99)	39560 (24.62)	104093 (28.02)	
Overweight + Obese	359710 (67.59)	113133 (70.41)	246577 (66.37)	
Overweight(<25)	86408 (16.24)	23219 (14.45)	63189 (17.01)	
Obese I (<30)	192223 (36.12)	53953 (33.58)	138270 (37.22)	
Obese II (≥30)	81079 (15.23)	35961 (22.38)	45118 (12.14)	
Waist circumference, cm	[n_w=526942]	[n_w=155759]	[n_w=371183]	
	87.22 ± 11.61	87.26 ± 12.78	87.2 ± 11.09	.952*
Hip circumference, cm	[n_w=531380]	[n_w=156434]	[n_w=374946]	
	93.55 ± 8.93	93.94 ± 9.62	93.39 ± 8.68	.480*
Systolic BP, mm Hg	[n_w=542807]	[n_w=162061]	[n_w=380746]	
	131.56 ± 22.34	122.41 ± 19.17	135.45 ± 22.47	<.001*
Diastolic BP, mm Hg	[n_w=542807]	[n_w=162061]	[n_w=380746]	
	82.31 ± 12.08	81.42 ± 12.23	82.69 ± 12.01	.188*
Present smoker	[n_w=511436]			.007†
No	405867 (79.36)	106245 (71.73)	299622 (82.47)	
Once a week	5813 (1.14)	2460 (1.66)	3353 (0.92)	
2-6 times a week	13127 (2.57)	4625 (3.12)	8501 (2.34)	
Every day	86630 (16.94)	34784 (23.48)	51846 (14.27)	
Ever smoked tobacco product	[n_w=511436]			<.001†
Not at all	415539 (81.25)	124187 (83.85)	291352 (80.19)	
Once a week	3118 (0.61)	584 (0.39)	2534 (0.7)	
2-6 times a week	6045 (1.18)	3719 (2.51)	2326 (0.64)	
Every day	57376 (11.22)	7135 (4.82)	50242 (13.83)	
Tried once	22156 (4.33)	9112 (6.15)	13044 (3.59)	
Occasionally	7202 (1.41)	3377 (2.28)	3825 (1.05)	
Ever consumed alcoholic beverage	[n_w=511436]			<.001†
No	219210 (42.86)	46397 (31.33)	172814 (47.56)	
Yes	172435 (33.72)	58747 (39.66)	113688 (31.29)	
Occasionally (during socials)	119791 (23.42)	42969 (29.01)	76821 (21.14)	
Binge drinker	[n_w=511436]			.052†
	64246 (12.56)	23116 (15.61)	41130 (11.32)	
General physical activity	[n_w=501646]			.078†
Low	248377 (49.51)	65458 (44.45)	182919 (51.62)	
High	253269 (50.49)	81819 (55.55)	171451 (48.38)	

Statistical tests used: * - Adjusted Wald test; † - Pearson's chi-squared test

Table 3. Dietary and biochemical profile of NNHeS adult respondents with diabetes

	All (n_w=546580)	Young Adults (n_w=162675)	Older Adults (n_w=383905)	*p*
		Mean ± SD		
Cereals and derivatives, g	[n_w=277580]	[n_w=78697]	[n_w=198883]	
	300.32 ± 148.29	339.2 ± 169.04	284.94 ± 136.5	.002
Rice and derivatives, g	[n_w=277580]	[n_w=78697]	[n_w=198883]	
	256.1 ± 151.51	284.32 ± 177.1	244.93 ± 138.85	.028
Corn and derivatives, g	[n_w=277580]	[n_w=78697]	[n_w=198883]	
	12.5 ± 48.33	17.54 ± 64.86	10.5 ± 39.91	.242
Other cereal products, g	[n_w=277580]	[n_w=78697]	[n_w=198883]	
	31.73 ± 37.02	37.34 ± 46.18	29.51 ± 32.51	.134
Starchy roots and tubers, g	[n_w=277580]	[n_w=78697]	[n_w=198883]	
	11.46 ± 39.39	6.14 ± 24.55	13.57 ± 43.74	.008
Sugar and syrups, g	[n_w=277580]	[n_w=78697]	[n_w=198883]	
	10.84 ± 18.68	11.46 ± 17.88	10.59 ± 19	.642
Dried beans/nuts/seeds, g	[n_w=277580]	[n_w=78697]	[n_w=198883]	
	7.8 ± 26.93	8.24 ± 24.14	7.63 ± 27.99	.821
Vegetables, g	[n_w=277580]	[n_w=78697]	[n_w=198883]	
	74.13 ± 82.16	66.75 ± 68.81	77.04 ± 86.8	.165

Table 3. Dietary and biochemical profile of NNHeS adult respondents with diabetes *(continued)*

	All (n_w=546580)	Young Adults (n_w=162675)	Older Adults (n_w=383905)	*p*
		Mean ± SD		
Green leafy and yellow, g	[n_w=277580] 29.45 ± 49	[n_w=78697] 26.23 ± 46.16	[n_w=198883] 30.72 ± 50.09	.314
Other vegetables, g	[n_w=277580] 44.68 ± 59.19	[n_w=78697] 40.52 ± 52.13	[n_w=198883] 46.32 ± 61.76	.319
Fruits, g	[n_w=277580] 41.92 ± 119.94	[n_w=78697] 32.18 ± 100.63	[n_w=198883] 45.78 ± 126.7	.193
Vitamin C rich fruits, g	[n_w=277580] 5.56 ± 30.41	[n_w=78697] 3.43 ± 20.83	[n_w=198883] 6.4 ± 33.43	.217
Other fruits, g	[n_w=277580] 36.36 ± 114.19	[n_w=78697] 28.75 ± 94.17	[n_w=198883] 39.38 ± 121.2	.282
Fish, meat, and poultry, g	[n_w=277580] 209.62 ± 147.03	[n_w=78697] 202.35 ± 139.85	[n_w=198883] 212.49 ± 149.89	.492
Fish and derivatives, g	[n_w=277580] 111.89 ± 112.83	[n_w=78697] 104.59 ± 91.22	[n_w=198883] 114.78 ± 120.31	.331
Meat and derivatives, g	[n_w=277580] 63.73 ± 87.88	[n_w=78697] 68.02 ± 89.65	[n_w=198883] 62.04 ± 87.26	.524
Poultry, g	[n_w=277580] 33.99 ± 57.46	[n_w=78697] 29.74 ± 52.95	[n_w=198883] 35.67 ± 59.15	.338
Eggs, g	[n_w=277580] 11.28 ± 19.71	[n_w=78697] 9.74 ± 16.63	[n_w=198883] 11.89 ± 20.79	.255
Milk and derivatives, g	[n_w=277580] 28.66 ± 84.57	[n_w=78697] 16.27 ± 41.66	[n_w=198883] 33.57 ± 96.02	.004
Whole milk, g	[n_w=277580] 16.89 ± 60.92	[n_w=78697] 8.71 ± 33.48	[n_w=198883] 20.12 ± 68.59	.008
Milk products, g	[n_w=277580] 11.78 ± 58.15	[n_w=78697] 7.56 ± 26.41	[n_w=198883] 13.45 ± 66.62	.159
Fats and oils, g	[n_w=277580] 5.81 ± 9.93	[n_w=78697] 5.34 ± 6.34	[n_w=198883] 5.99 ± 11.03	.436
Miscellaneous, g	[n_w=277580] 48.41 ± 134.49	[n_w=78697] 42.59 ± 103.49	[n_w=198883] 50.71 ± 145	.508
Beverages, g	[n_w=277580] 42.36 ± 129.89	[n_w=78697] 37.42 ± 103.17	[n_w=198883] 44.32 ± 139.13	.569
Condiments and spices, g	[n_w=277580] 1.7 ± 4.16	[n_w=78697] 1.48 ± 3.26	[n_w=198883] 1.8 ± 4.47	.406
Other miscellaneous, g	[n_w=277580] 4.34 ± 38.17	[n_w=78697] 3.7 ± 17.3	[n_w=198883] 4.6 ± 43.78	.740
Total calcium, g	[n_w=277580] 329.9 ± 250.39	[n_w=78697] 315.83 ± 224.42	[n_w=198883] 335.47 ± 260.06	.372
Total carbohydrates, g	[n_w=277580] 280.62 ± 117.5	[n_w=78697] 306.13 ± 142.16	[n_w=198883] 270.53 ± 104.74	.014
Total energy, kcal	[n_w=277580] 1647.88 ± 679.08	[n_w=78697] 1776.78 ± 758.38	[n_w=198883] 1596.88 ± 639.16	.023
Total fats, g	[n_w=277580] 31.49 ± 25.78	[n_w=78697] 34.27 ± 26.9	[n_w=198883] 30.39 ± 25.28	.175
Total iron, mg	[n_w=277580] 8.35 ± 4.06	[n_w=78697] 8.65 ± 4.02	[n_w=198883] 8.23 ± 4.08	.338
Total vitamin C, mg	[n_w=277580] 18.39 ± 8.27	[n_w=78697] 18.92 ± 9.07	[n_w=198883] 18.18 ± 7.93	.423*
Total protein, g	[n_w=277580] 55.78 ± 24.3	[n_w=78697] 58.35 ± 26.34	[n_w=198883] 54.77 ± 23.41	.185
Total niacin, mg	[n_w=277580] 0.74 ± 0.66	[n_w=78697] 0.73 ± 0.56	[n_w=198883] 0.74 ± 0.69	.794
Total riboflavin, mg	[n_w=277580] 0.95 ± 3.53	[n_w=78697] 0.89 ± 0.55	[n_w=198883] 0.97 ± 4.16	.694
Total vitamin A, mcg RE	[n_w=277580] 524.05 ± 1403.39	[n_w=78697] 434.43 ± 923.12	[n_w=198883] 559.52 ± 1552.61	.326
Total thiamine, mg	[n_w=277580] 524.05 ± 1403.39	[n_w=78697] 434.43 ± 923.12	[n_w=198883] 559.52 ± 1552.61	.319
UIE, µg/dL	[n_w=512745] 115.48 ± 116.6	[n_w=146345] 139.32 ± 131.71	[n_w=366400] 105.96 ± 108.65	.001
Vitamin A, µg/dL	[n_w=543068] 48.48 ± 17.94	[n_w=161535] 48 ± 16.89	[n_w=381533] 48.68 ± 18.37	.608
Hemoglobin, g/dL	[n_w=545606] 14.04 ± 1.54	[n_w=162229] 14.47 ± 1.58	[n_w=383377] 13.86 ± 1.48	<.001

Means were compared by adjusted Wald test

Table 4. Cardiometabolic risk factors among Young and Older Adults with diabetes

	Total (n_w=546580)	Young Adults (n_w=162675)	Older Adults (n_w=383905)	*p*
	Frequency (%); Mean ± SD			
Metabolic syndrome				
High waist circumference [n_w=526942]	310582 (58.94)	85428 (54.85)	225153 (60.66)	.135
Male (≥90cm) [n_w=256605]	101765 (39.66)	33052 (36.64)	68713 (41.3)	.362
Female (≥80cm) [n_w=270338]	208817 (77.24)	52376 (79.92)	156441 (76.39)	.444
TG ≥150 mg/dL [n_w=545353]	356040 (65.29)	106515 (65.73)	249524 (65.1)	.305
Low HDL-C [n_w=545353]	451071 (82.71)	135401 (83.56)	315670 (82.35)	.667
Male (<40 mg/dL) [n_w = 261488]	192816 (73.74)	68665 (75.96)	124152 (72.56)	.447
Female (<50 mg/dL) [n_w = 283864]	258255 (90.98)	66736 (93.15)	191518 (90.25)	.328
BP ≥130/85 mmHg [n_w = 542807]	302461 (55.72)	61263 (37.8)	241198 (63.35)	<.001
Metabolic syndrome [n_w = 546580]	457841 (83.76)	131983 (81.13)	325859 (84.88)	.194
Lipid profile				
TC to HDL-C ratio	[n_w= 545353]	[n_w=162041]	[n_w=383311]	
	7.62 ± 4.86	7.76 ± 6.03	7.56 ± 4.28	.651
Total cholesterol-to-HDL cholesterol ratio ≥ 5.9	308712 (56.61)	86107 (53.14)	222605 (58.07)	.201
Total cholesterol, mg/dL	[n_w= 545353]	[n_w=162041]	[n_w=383311]	
	224.82 ± 54.14	215.46 ± 51.15	228.77 ± 54.91	.002
TC ≥200 mg/dL	373490 (68.49)	102872 (63.48)	270618 (70.6)	.051
LDL-C, mg/dL	[n_w= 544790]	[n_w=161479]	[n_w=383311]	
	146.57 ± 46.9	136.24 ± 44.89	150.92 ± 47.08	<.001
LDL-C above cutoff	[n_w= 544790]	[n_w=161479]	[n_w=383311]	
≥100 (vs <100)	462729 (84.94)	133308 (82.55)	329421 (85.94)	.227
≥ 70 (vs <70)	521547 (95.73)	151585 (93.87)	369962 (96.52)	.119
≥55 (vs <55)	536321 (98.45)	157226 (97.37)	379095 (98.9)	.193
HDL	[n_w= 545353]	[n_w=162041]	[n_w=383311]	
	34.84 ± 12.52	34.19 ± 12.07	35.12 ± 12.71	.316
VLDL ≥130 [nw=545353]	11636 (2.13)	6392 (3.94)	5243 (1.37)	.052
Triglycerides	[n_w= 545353]	[n_w=162041]	[n_w=383311]	
	217.52 ± 134.6	227.99 ± 157.58	213.09 ± 123.49	.174
BMI, kg/m^2	[n_w=532210]	[n_w=160675]	[n_w=371535]	
Underweight (<18.5)	28848 (5.42)	7982 (4.97)	20866 (5.62)	.696
Normal (<23)	143652 (26.99)	39560 (24.62)	104093 (28.02)	.315
Overweight (<25)	86408 (16.24)	23219 (14.45)	63189 (17.01)	.257
Obese I (<30)	192223 (36.12)	53953 (33.58)	138270 (37.22)	.089
Obese II (≥30)	81079 (15.23)	35961 (22.38)	45118 (12.14)	.002
Waist–hip ratio				
Male	[n_w=256604]	[n_w=90220]	[n_w=166384]	
	0.94 ± 0.07	0.93 ± 0.08	0.95 ± 0.07	.059
Male ratio >0.90	187269 (72.98)	57997 (64.28)	129272 (77.69)	.005
Female	[n_w= 270338]	[n_w=65538]	[n_w=204799]	
	0.92 ± 0.07	0.92 ± 0.06	0.92 ± 0.07	.825
Female ratio >0.80	261993 (96.91)	63167 (96.38)	198826 (97.08)	.731
Waist–height ratio				
Waist–height ratio >0.5	402334 (76.35)	110422 (70.89)	291912 (78.64)	.023
Male	[n_w=256604]	[n_w=90220]	[n_w=166384]	
	0.53 ± 0.07	0.52 ± 0.08	0.54 ± 0.07	.095
Male ratio >0.50	170336 (66.38)	53104 (58.86)	117232 (70.46)	.023
Female	[n_w= 270338]	[n_w=65538]	[n_w=204799]	
	0.58 ± 0.07	0.59 ± 0.08	0.57 ± 0.07	.236
Female ratio >0.50	231998 (85.82)	57318 (87.46)	174681 (85.29)	.570
Blood pressure				
Normal (SBP<120 AND DBP<80)	125979 (23.35)	57907 (35.73)	68071 (18.03)	-
Elevated (SBP 120-129 AND DBP <80)	37378 (6.93)	9353 (5.77)	28025 (7.42)	<.001
HTN stage 1 (SBP 130-139 OR DBP 80-89)	152312 (28.23)	51768 (31.94)	100544 (26.63)	<.001
HTN stage II (SBP≥140 OR DBP≥90)	223966 (41.5)	43034 (26.55)	180932 (47.92)	.004
Behavior				
Current drinker [n_w = 511436]	218456 (42.71)	83686 (56.5)	134770 (37.09)	<.001
Alcohol intake in the last 30 days among current drinkers (14.0g alcohol/ standard drink)	[n_w= 204827]	[n_w=77790]	[n_w=127037]	
	4.40 ± 7.48	3.7 ± 5.49	4.82 ± 8.46	.138
Current smoker [n_w = 511436]	105570 (20.64)	41869 (28.27)	63701 (17.53)	.002
Low physical activity [n_w = 501646]	253269 (50.49)	81819 (55.55)	171451 (48.38)	.077
Unhealthy diet (<400g of fruits and vegetables per day) [n_w = 277580]	227334 (81.9)	68209 (86.67)	159125 (80.01)	.066

Table 5. Risk factors associated with Metabolic Syndrome (MetS) among young adults with diabetes mellitus

	Total (n_w=162675)	With Metabolic Syndrome (n_w=131983)	No Metabolic Syndrome (n_w=30692)	Crude Odds Ratio (95% CI)	*p*
	Frequency (%); Mean ± SD; Median (Range)				
Age, y	36.79 ± 6.4	37.22 ± 6.26	34.94 ± 6.75	1.054 (1.01–1.1)	.029
Sex					
Male	91027 (55.96)	70183 (53.18)	20845 (67.92)	1.0 (Reference)	-
Female	71648 (44.04)	61800 (46.82)	9847 (32.08)	1.864 (0.92–3.76)	.082
Total fat intake, g					
	34.27 ± 26.9	36.07 ± 28.98	26.94 ± 14.11	1.016 (1.0004–1.03)	.044
	26.26 (1.27–162.98)	26.7 (1.27–162.98)	23.95 (6.8–57.12)		
General physical activity					
Low	65458 (44.45)	57365 (47.36)	8093 (30.94)	2.009 (0.93–4.32)	.074
High	81819 (55.55)	63752 (52.64)	18067 (69.06)	1.0 (Reference)	-
Current drinker [n_w = 148113]	83686 (56.5)	69862 (58.03)	13824 (49.88)	1.389 (0.7–2.78)	.351
Alcohol intake in the last 30 days among current drinkers (14.0 g alcohol per standard drink)	n_w = 77790	n_w = 64422	n_w = 13370		
	3.70 ± 5.49	3.35 ± 5.25	5.40 ± 6.37	0.942 (0.87–1.01)	.114
	0.84 (0 to 20)	0.84 (0 to 20)	3.30 (0 to 20)		
Current smoker [n_w = 148113]	41869 (28.27)	32803 (27.25)	9065 (32.71)	0.77 (0.37–1.62)	.489
Civil status					
Single	39844 (24.49)	29887 (22.64)	9957 (32.44)	1.0 (Reference)	-
Married	106697 (65.59)	88300 (66.9)	18397 (59.94)	1.599 (0.76–3.36)	.215
Live-in	12459 (7.66)	11399 (8.64)	1060 (3.45)	3.582 (0.72–17.84)	.119
Widowed	1285 (0.79)	1285 (0.97)	0	-	-
Separated	2390 (1.47)	1112 (0.84)	1278 (4.17)	0.29 (0.04–2.39)	.249
Highest educational attainment					
No or incomplete primary education	13478 (8.29)	9508 (7.2)	3969 (12.93)	-	-
Elementary graduate	19040 (11.7)	14845 (11.25)	4195 (13.67)	1.913 (0.72–5.09)	0.193
Some high school education	22763 (13.99)	16042 (12.15)	6721 (21.9)	1.602 (0.76–3.36)	0.212
High school graduate	46601 (28.65)	35985 (27.26)	10616 (34.59)	2.135 (1.09 to 4.18)	0.027
Vocational or technological course	11134 (6.84)	11134 (8.44)	0	3.576 (1.59 to 8.05)	0.002
Some college education	18702 (11.5)	16239 (12.3)	2463 (8.02)	2.462 (1.09 to 5.57)	0.031
College graduate	30148 (18.53)	27420 (20.78)	2728 (8.89)	2.738 (0.98 to 7.65)	0.055
Postgraduate	406 (0.25)	406 (0.31)	0	-	-
Wealth quintile	n_w=159846	n_w=129154	n_w=30692		
Poorest	24791 (15.51)	18599 (14.4)	6191 (20.17)	1.0 (Reference)	-
Poor	23003 (14.39)	18329 (14.19)	4674 (15.23)	1.305 (0.44–3.88)	.631
Middle	31221 (19.53)	23700 (18.35)	7521 (24.5)	1.049 (0.38–2.92)	.927
Rich	36838 (23.05)	30333 (23.49)	6506 (21.2)	1.552 (0.55–4.34)	.401
Richest	43993 (27.52)	38193 (29.57)	5800 (18.9)	2.192 (0.75–6.44)	.152
Total energy, kcal	n_w = 78697	n_w = 63123	n_w = 15574		
	1776.78 ± 758.38	1810.67 ± 787.07	1639.38 ± 624.54	1.0003 (0.9997–1.0009)	.298
	1654.34 (573.27 to 3824.62)	1748.45 (573.27 to 3824.62)	1406.08 (906.64 to 3047.99)		
Total carbohydrates, g	n_w = 78697	n_w = 63123	n_w = 15574		
	306.13 ± 142.16	309.07 ± 145.34	294.19 ± 130.64	1.0008 (0.997–1.004)	.653
	271.53 (87.47 to 685.38)	271.53 (87.47 to 685.38)	248.54 (98.99 to 582.84)		
Total protein, g	n_w = 78697	n_w = 63123	n_w = 15574		
	58.35 ± 26.34	59.23 ± 27.41	54.76 ± 21.58	1.007 (0.99–1.02)	.407
	52.82 (13.57 to 156.28)	52.82 (13.57 to 156.28)	52.89 (25.63 to 118.17)		

Statistical test used: Logistic regression

current smokers (28% vs 18%); but better risk profiles in terms of hypertension stage 1 (15% vs 28%), hypertension stage 2 (11% vs 19%), total cholesterol (215.46±51.15 mg/dL vs 228.77±54.91 mg/dL, LDL-C (136.24±44.89 mg/dL vs 228.77±54.91 mg/dL), and BP ≥130/85 mmHg (38% vs 63%). When using WHR and WHtR for obese classification, male older adults have greater proportions with central obesity than young adults (77% vs 64%).

Among the young adults with diabetes, age and total fat intake were shown to be factors predictive of metabolic syndrome (Table 5). With every unit increase in age and total fat intake, the odds of having metabolic syndrome were raised by 5.4% (95% CI 1%–10%) and 1.6% (95% CI 0.04%–3%), respectively. Being at least a high school graduate has increased odds of having metabolic syndrome (cOR 2.135, 95% CI 1.09 to 4.18).

DISCUSSION

This study shows that young-onset diabetes in Filipinos have some differences in cardiometabolic risk factors compared to late-onset diabetes. However, these two age categories have similarly high percentages of the metabolic syndrome. In this national cross-sectional survey from 2013, one in five Filipino adults with diabetes was diagnosed before age 45 years, with prevalence of 2.64% which is low compared to published literature in other countries. The 2013 national survey in China, determined the prevalence of diabetes in the 20- to 39-year age-group to be 5.9%,[6–8] in

Hong Kong, it is at 21.3% of the DM cohort,[9] while the Joint Asia Diabetes Association (JADE) program which looked into Asian population reported around 18% young onset DM prevalence. Filipino young-onset diabetes had mean age of 37 years, mostly males, married, and employed. Similar to what was published in Asian YOD, mean age at diagnosis is 33 years, comprising mostly of men, and are obese.[3] In the US, YOD was diagnosed at 36 years old, with 7.7 years duration of diabetes, mostly in non-Hispanic Black population, and twice likely to be more obese than their young non-diabetic counterparts.[4] In Indians, the onset of YOD is nearly 10 years earlier than what was observed in other Asian countries, and 20 years earlier than what is usually observed in the Caucasian population, with T1DM and T2DM occurring with equal frequency at 40%.[10]

Filipino YODs were noted to be heavier, with higher BMIs and classify as being Obese II. This result was similar in a survey enrolling 41,029 patients with T2D across Asia.[3] A predominance of overweight and obese class was also seen among individuals with type 1, youth-onset type 2 and monogenic diabetes in the US, Germany and Austria making it difficult to make clinical distinctions.[11] Since we see that obesity is a consistent feature, it is interesting to note that there is a similar proportion of diabetics being physically active among the young and the old (55% vs 48%, p=0.078), yet there are more obese persons among the YOD (56% vs 49%, p=0.01). Although not significantly different, both the YOD and LOD are more than 80% unhealthy eaters. The mix between the nonmodifiable— genetics, race/ethnic background, family history of diabetes, being the offspring of a pregnancy complicated by gestational diabetes mellitus (GDM); and modifiable—poor diet, disordered eating behaviors, stress, and depression are identified contributors to this growing problem of obesity.[12,13]

When using the WHR and WHtR criteria to diagnose obesity, more male older adults with diabetes were classified as obese. This represents the population which may have a normal BMI but with central obesity. Central obesity means increased visceral adipose tissue (VAT) which has been associated with a range of metabolic abnormalities, including insulin resistance and adverse lipid profiles— the known risk factors for T2D and CVD.[14] VAT has been shown to be involved in activating pro-inflammatory cytokines, oxidative stress and the renin–angiotensin–aldosterone system (RAAS). Cut-off values used for WHR were 0.90 for men and 0.80 for women based on WHO Asian cutoffs also served as the basis in multiple Asian studies showing Asians to have an increased metabolic risk at lower waist circumference, and lower waist–hip ratio than Europeans. Waist-height ratio as a measure of abdominal obesity was also shown to be better than BMI in predicting CV risks.[15-16] In a study done among Filipinos living in rural areas, cardiometabolic diseases occurred at lower BMI, waist circumference, and WHR cut-offs compared to WHO recommendations. Obesity cut-offs in rural Filipino males and females are BMI of 24 and 23 kg/m^2, WC of 84 and 77 cm, and WHR 0.91 and 0.85, respectively. Countries in the Western Pacific and Southeast Asia also exhibited lower cut-offs for at least one cardiometabolic disease to occur.[17] Studies in Filipino-American women showed that those of the same age and sex with the same BMI have a higher fat percentage; thus, at higher risk for diabetes, high blood pressure, and heart disease compared to Caucasians.[18] It is important to screen for obesity using these more sensitive indices as the risk of T2D and diabetic complications increases continuously with increasing obesity.[19]

More Filipino YOD are current smokers. Smoking has been identified as a risk factor for diabetes in the young, increasing the odds by 1.6 fold. The exact mechanism on how smoking causes diabetes is still under study, however the theory involves directly damaged β-cell function, increased inflammation and oxidative stress, and impaired endothelial function.[20] Epidemiologic studies such as the European Investigation into Cancer (EPIC-Norfolk) showed cigarette smoking was independently associated with higher hemoglobin A1c (HbA1c) concentrations, with both male and female smokers exhibiting similar changes in HbA1c values.[21] The Health Professionals' Follow-Up Study showed men who smoked 25 or more cigarettes per day had a relative risk of incident diabetes of 1.94 (95%CI 1.25, 3.03) compared to non-smokers.[22] Furthermore, smoking was associated with an increased risk for diabetes treatment, hospitalization, and mortality among both men and women, and risk increased in a dose-response dependent manner with the number of cigarettes smoked per day, with men being affected more than women.[23] A cause-effect relationship between smoking and diabetes cannot be solidly established as it is multifactorial. Stress, diet, levels of physical activity and distribution of body fat are confounders in the analysis from various studies. Active smoking was seen as a risk factor for progression of diabetic nephropathy, retinopathy and neuropathy with a dose-dependent risk increase among smokers with T1D, while significantly decreased incidence of retinopathy in smokers with T2D.[24] It has also been shown that smoking is one of the key risk factors for cardiovascular disease and the strongest predictor of death.[25] Unfortunately for the NNHeS, no data on health seeking behaviors were sought and no inclusion of measures of kidney function nor micro/macroalbuminuria, retinopathy, neuropathy, and no hard cardiovascular endpoints were noted.

Alcohol consumption is also a relevant lifestyle factor in the development of T2D. Significantly more YOD are current drinkers at 40% vs 31% in older adults, and alcohol use has been found to be a risk factor to develop DM at an early age. Various trials have shown that moderate alcohol consumption improved insulin sensitivity by increasing an anti-inflammatory plasma protein—adiponectin, while some studies showed an opposite effect.[26,27] The difference in results might be a variable of quantity, the type of alcoholic beverage, and drinking patterns. Twenty prospective cohort studies included in a meta-analysis showed that compared with non-alcohol beverage drinkers, the relative risk (RR) for T2D among men was most protective when consuming 22 g/day alcohol (RR 0.87 [95% CI 0.76–1.00]) and became deleterious at just over 60 g/day alcohol (1.01 [0.71–1.44]), and among women, consumption of 24 g/day alcohol was most protective (0.60 [0.52–0.69]) and became deleterious at about 50 g/day alcohol (1.02 [0.83–1.26]).[28] Among 70,551 Danish subjects, the lowest risk of diabetes was observed at 14 drinks/week in men (HR 0.57 [95% CI 0.47, 0.70]) and at 9 drinks/week in women (HR 0.42 [95% CI 0.35, 0.51]), relative to no alcohol intake, and consumption of alcohol on 3–4 days weekly was associated

with significantly lower risk for diabetes in men (HR 0.73 [95% CI 0.59, 0.94]) and women (HR 0.68 [95% CI 0.53, 0.88]), compared to binge drinking at once a week.[29] Alcohol consumption in this study composed of 3-4 standard drinks per day (40-60 g alcohol) for the young and old cohorts, which is above the recommended amount of alcohol intake per day. Binge drinking status comprised 12% of the population with diabetes with similar frequencies between groups. However, no correlation was established between binge drinking and having early onset DM.

The diets of YOD are characterized by higher cereal and rice intake, lower intake of starchy roots and tubers, lower intake of milk and derivatives and whole milk, higher total carbohydrate intake, and higher total energy intake compared with their older counterpart. The average total caloric and carbohydrate intakes were higher in the young adult group than in the older adult group. Carbohydrate intake for both groups were above the suggested intake of 60% total caloric requirement. This pattern of eating behavior, dietary abundance, coupled with sedentary lifestyle may affect weight. Chronic excess caloric intake leads to excessive weight gain then obesity, which has been shown as the driver of insulin resistance fueling early onset diabetes. Those with unhealthy dietary habits, including intake of simple sugars, low dietary quality, skipping meals, and binge eating predispose to glucose spikes and hyperinsulinemia.[30] Sustained hyperinsulinemia increases risk for early β cell exhaustion and cardiometabolic diseases.[31] Glucose dysregulation seen in both young adult and pediatric populations shows changes in the distribution of fat. A combination of high intramyocellular lipid content, increased VAT, decreased subcutaneous and ectopic liver fat deposition and increased epicardial adipose tissue was noted.[14,32] Mechanisms from disorders in lipid metabolism and inflammation also support the development of diabetes mellitus in the young. Obese individuals have chronically increased levels of circulating free fatty acids, and might contribute to increased reactive oxygen species and impaired insulin secretion. Pro-inflammatory factors namely tumor necrosis factor-α, interleukin 1β, and high-sensitivity C-reactive protein are noted to be increased in hyperglycemic states.[33] Physiologic changes that occur with aging may be contributory to the observed decreased caloric intake among the LOD. Swallowing problems, satiety issues, indigestion and mechanical problems as a result of a more advanced age may hinder food intake.[34] Thus, issues of malnutrition and hypoglycemia may be more encountered in the older cohort than in YOD.

Urinary iodine excretion and hemoglobin were significantly greater in the YOD than in LOD. Majority of iodine absorbed by the body is excreted in the urine, so urinary iodine excretion is considered as a sensitive indicator of iodine intake and changes in iodine status.[35] A high UIE rate may indicate sufficient iodine intake, or in excessively high levels, hyperthyroidism. Higher UIE rates were observed in the young adults, indicating higher iodine intake in this group. It is interesting to note, however, that when the dietary sources of each age group is considered, the older adults had more intake of iodine-rich foods, such as seafood and dairy products. In addition, young adults were noted to have a lower prevalence of diabetes. In a study conducted in Saudi Arabia which is an iodine-sufficient country, patients with diabetes have lower iodine concentration,[36] which may implicate that UIE levels can be an indicator of insulin resistance or glucose control. Consequently, persons with diabetes may also benefit from routine urinary iodine determination to screen for thyroid dysfunction.[37] Hemoglobin, meanwhile, was significantly greater in the younger adult bracket. This is consistent with age-related decrease in hemoglobin levels, affected by physiologic causes, chronic diseases, nutritional deficiencies, or changes in diet. In contrast, levels of vitamin A were comparable between the two. Vitamin A, an antioxidant vitamin, is present in animal products such as organ meats, fish, egg yolks, and fortified milk. Although comparable between the two age groups, vitamin A ranks among the nutrients with the highest level of inadequacy in Filipino adults. The prevalence of inadequacy of vitamin A also increases significantly with age.[38] The importance of vitamin A in pancreatic β cell development is highlighted by decreased β cell mass and impaired glucose tolerance in vitamin A-deficient adult mice. Reduced β cell mass increased α cell mass, with hyperglycemia and altered serum insulin and glucagon profiles.[39] Furthermore, micronutrient deficiency may also be related to developing macro and microvascular complications of diabetes, as diabetic patients with vitamin A deficiency are also seen to develop nonhealing foot ulcers.[40] The continued high prevalence of diabetes in both young and older age groups in the Philippines suggests a complex interplay of many different factors leading to its development. The different common nutrients found inadequate in the typical Filipino diet may be contributing to this burden.

The prevalence of hypertension is lower in YOD. Hypertension was also not found to be a cardiometabolic risk factor in the Filipino YOD cohort. Although this may reflect a subclinical disease activity because of a younger age of subjects, this study is limited by a one-point sampling, and no follow-ups. This result is in contrast to those seen in some studies that at diagnosis, 26% of adolescents with diabetes have hypertension, increasing to 50% by the fourth decade.[41] The Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) clinical trial involving 699 adolescents from 10-17 years old with newly diagnosed T2D showed 11.6% were hypertensive at baseline and 33.8% by end of study with average follow-up of 3.9 years, with male sex and higher BMI significantly increasing the risk for hypertension and eventually nephropathy.[42] Analysis from the Framingham study showed that patients with coexisting T2D and hypertension had higher rates of mortality from all causes (32 vs 20 per 1000 person-years; $p<0.001$) and cardiovascular events (52 vs 31 per 1000 person-years; $p<0.001$) compared with normotensive subjects with DM.[43] Among the young, it is safe to infer that prolonged disease exposure of the YOD with hypertension can significantly lead to earlier and poorer cardiovascular and metabolic profiles. Implications of this would entail early aggressive glycemic, cardiometabolic and BP control for the young patients with diabetes.

The lipid profiles of Filipino YOD were similar to LOD with high TC, high LDL, high Tg, and low HDL. However, YOD had significantly lower TC and LDL compared to older adults with diabetes. Mean LDL between YOD and LOD population is high at 136 and 150 mg/dl, with mean TC at 215 and 228 mg/dl, respectively, which is above the recommended treatment targets. Only 6% and 4% had

desirable LDL of <70 mg/dl among the YOD and LOD. This is in contrast to the published Asian YOD data where mean LDL was lower at 107 vs 106 mg/dl, and also mean TC was lower at 184 vs 181 mg/dl, respectively.[3] With the presence of obesity in the YOD, we expect a proportional increase of TC and LDL. The mechanism for this has not been truly established but the hypothesis is still related to insulin resistance. HbA1c was significantly directly related to TC and non-HDL (calculated as the TC minus HDL).[44] In this study, more than 90% of females and 70% of males had low HDL, with mean of 34 mg/dl in the YOD, and 35 mg/dl in LOD. This is consistent with previously published data on Filipinos having lower HDL (40.8±0.2 mg/dL) compared in NHANES (60.7±0.7 mg/dL), although there is a phenomenon of isolated low HDL-c phenotype.[45] Triglyceride values were also elevated to more than 200 mg/dl for both groups. The lipid profile picture of young diabetics are typical and comparable to those with LOD. What is alarming is that if this picture gives us a glimpse of glycemic control which suggests poorly controlled diabetes, and foreseen cardiovascular effects of dyslipidemia. However we cannot commit to the relation of blood sugar control in our Filipino young DM cohort as no HbA1c was taken for the patients included in the national survey.

More than 80% of the YOD and LOD have metabolic syndrome. The absence of significant differences in the proportion of metabolic syndrome between the YOD and LOD has important implications. Early onset presence of MetS predispose to around 2.5 fold increased atherosclerotic cardiovascular disease, and five-fold increased diabetic complications which are major contributors to morbidity and mortality all over the world.[46] Metabolic syndrome also affects early cognitive decline and early onset dementia.[47] Several studies have shown that MetS prevalence increases with age. Clustering of the metabolic syndrome regardless of components, happened at 45-65 years of age, and decreased by >65 year old.[48] However, in the Filipino population MetS starts early on. Both YOD and LOD had increased prevalence for metabolic syndrome, with individual components not significantly different in proportion between the young and the old except for higher hypertension prevalence in the old. The overall MetS prevalence in the general population according to the 2013 Philippine NNHeS is 27%, and ranged from 12-19% in an earlier local study.[49] Having diabetes increases MetS prevalence as what was shown in this study, obesity in the young is another factor contributing to MetS to a greater extent than in the elderly, and may account for the observed increase in prevalence of MetS in recent years that is disproportionately highest in the young.[50] Similarly, it is demonstrated in this study that increasing age and fat intake were identified to be predictors for the occurrence of MetS among Filipino YOD. Thus, for prevention, we need to institute targeting a healthy diet and achieving a desirable weight in the young.

Type 2 diabetes mellitus has been previously known as a disease of older adults but the overall burden for young-onset diabetes continues to increase as declines in mortality rates among people with diabetes have been seen during the past two decades in every age-group except young adults aged 20–44 years.[4] A better understanding of the cardiometabolic characteristics of this population is important, to render effective service delivery and timely preventive mechanisms to halt development of chronic diabetic complications. Interventions should be multifaceted to address multiple barriers in diabetes care.

Limitations

These are some of the limitations of this study: (1) there was no distinction as to the type of diabetes; (2) young-onset diabetes diagnosis was based on age alone at the time of the survey rather than based on the onset of the diabetes with no way to verify if these are undiagnosed DM rather than already with ongoing treatment; (3) diabetic complication end points were not investigated i.e. retinopathy, neuropathy, nephropathy, macrovascular complications- stroke, myocardial infarction; (4) diabetes control was not reported, and (5) no follow-up studies were conducted.

Strengths of this study include that it is a nationally representative data from the Philippines, and cardiometabolic risk factors were obtained through standardized measurement and laboratory procedures.

CONCLUSIONS

Early-onset diabetes mellitus appears to be driven by obesity, MetS and social behaviors. More YOD were obese despite being more physically active. YOD are also unhealthy eaters. Young Filipino adults were more likely to have diabetes when they are obese, smokers, and alcoholic beverage drinkers, while the occurrence of MetS is affected by increasing age, excess fat intake and advanced educational attainment. The similarly high prevalence of metabolic syndrome in both the YOD and LOD has important implications on the early development of atherosclerotic cardiovascular diseases and diabetic complications. These findings suggest a need for more precise screening, management, and prevention strategies to decrease diabetes and MetS risk. Future directions of this work include the need for a prospective study to further investigate the relationship between the risk factors, as this can lead to an increased disease prevalence and mortality rate among YOD as they age.

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Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

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ORIGINAL ARTICLE

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Prevalence of Insulin Receptor Substrate-1 Gene (G972R) Polymorphism, Insulin Resistance, and Determination of β-Cell Function among Overweight and Obese Persons with Type 2 Diabetes Mellitus

Thae Nu Htwe,[1] Ohnmar Myint Thein,[1] Saw Wut Hmone,[2] Myat Thandar[3]

[1]*Department of Physiology, University of Medicine 2, Yangon, The Republic of the Union of Myanmar*
[2]*Department of Pathology, University of Medicine 1, Yangon, The Republic of the Union of Myanmar*
[3]*University of Nursing, Yangon, The Republic of the Union of Myanmar*

Abstract

Background. Type 2 diabetes mellitus (T2DM) is the most common metabolic disorder and its pathogenesis is characterized by a combination of peripheral insulin resistance and impaired insulin secretory capacity of pancreatic β cell. Genetic predisposition interacts with environmental factors including diet, physical activity, and age leading to the development of diabetes.

Objective. To determine the proportion of overweight and obese persons with type 2 diabetes and to compare the fasting blood sugar, fasting serum insulin, insulin resistance and β-cell function in G972R carrier and non-carrier overweight and obese persons with type 2 diabetes.

Methodology. One hundred overweight and obese patients with T2DM were recruited from persons with diabetes attending the Diabetes Outpatient Department of Yangon General Hospital. History taking and physical examination were done and blood samples were collected. Plasma glucose level was determined by the glucose oxidase method and fasting serum insulin was measured by enzyme linked immunoassay (ELISA) kit method. Polymerase chain reaction and Restriction Fragment Length Polymorphism were done for genetic polymorphism.

Results. Among 100 overweight and obese subjects with T2DM, 81 patients were of homozygous (G/G) genotype, 18 patients were of heterozygous (G/A) and only one patient of homozygous (A/A) genotype. There was no statistically significant difference in the proportion of genotypes between overweight and obese subjects with T2DM.

There was no significant difference in fasting blood sugar (FBS), fasting serum insulin, HOMA-IR, β-cell function, lipid parameters between IRS-1 (G972R) carriers and non-carriers. There is significant negative correlation between insulin resistance and TG level (r^2=0.0529, p=0.01).

Conclusion. It was concluded that IRS-1 G972R polymorphism was not important in insulin resistance, β-cell function and lipid parameters in overweight and obese T2DM. There could be a number of candidate genes in the pathophysiology of diabetes mellitus, genetic sequencing of IRS-1 and other genes in the insulin signaling pathway, and finding out the alteration in their genetic patterns would provide clues for the association of the site-specific polymorphisms of these genes with insulin resistance in T2DM.

Key words: IRS-1, insulin resistance, β-cell function, lipid profile

INTRODUCTION

Diabetes mellitus is now declared as a global epidemic.[1] World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes, according to 2005 figures.[2] This number is likely to be more than double by 2030 without intervention. According to WHO estimation, the prevalence of diabetes mellitus in Myanmar was 2.4% in 1995 and it will be 3.2 % in the year 2025.[3]

Insulin receptor substrate-1 (IRS-1) occupies a key position in the insulin signaling pathway.[4] As IRS-1 is the first substrate in this cascade, an impaired IRS-1 function may result in a defect in insulin signaling.[5] Thus genetic changes in IRS-1 may potentially contribute toward the development of insulin resistance, the most common of these being a glycine to arginine change at codon 972 (G972R).[6]

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Corresponding author: Prof. Thae Nu Htwe, MBBS, MMedSc, PhD (Physiology)
Professor, Department of Physiology, University of Medicine 2,
Yangon, The Republic of the Union of Myanmar
E-mail: thaenushinthant @gmail.com
ORCiD: https://orcid.org//0000-0003-1049-0704

The prevalence of IRS-1 (G972R) polymorphism was higher in type 2 diabetes mellitus (T2DM) especially in obese patients, and the prevalence of polymorphism is reported to be varied in various studies probably due to differences in genetics, race and ethnicity. There are also conflicting reports regarding the relationship between the IRS-1 (G972R) polymorphism and insulin resistance, fasting plasma insulin and blood glucose control. The insulin sensitivity or pancreatic β-cell function did not differ between carrier and wild type subjects in non-obese patients but showed significant difference in obese patients.[7] Jellema et al., showed that differences in fasting insulin and homeostatic model assessment-insulin resistance (HOMA-IR) between carriers and non-carriers were more pronounced and significant in obese subjects, but not in the non-obese subjects.[5] Analysis of variance also showed a significant interaction between the heterozygous forms of the codon 972 variant and obesity. In addition, the IRS-1 (G972R) polymorphism is associated with insulin resistance. The proportion of carriers was higher in T2DM patients with either insulin resistance or dyslipidemia.[8]

Genetic data of IRS-1 (G972R) polymorphism in Myanmar is not available yet, so this is a preliminary study. Since the prevalence of IRS-1 (G972R) polymorphism in Myanmar might differ from other regions and the effect of polymorphism on insulin resistance (IR) in T2DM subjects is not yet reported, the prevalence of polymorphism and the association of polymorphism with insulin resistance, β-cell function, and lipid parameters were investigated in the present study. The findings of the present study would highlight the genetic variation of polymorphism in T2DM among populations and show whether the IRS-1 variant has effect on the insulin resistance and lipid parameters particularly in overweight and obese individuals.

METHODOLOGY

Hundred overweight and obese patients with T2DM were recruited from persons with diabetes attending the Diabetes Outpatient Clinic of Yangon General Hospital according to inclusion and exclusion criteria. Inclusion criteria were patients diagnosed with T2DM who are taking Metformin only, age over 40 years and BMI ≥25 kg/m^2. Excluded in the study were T2DM patients taking oral hypoglycemic drugs other than Metformin and T2DM patients with pregnancy.

History taking and physical examination including anthropometric measurement were done and blood samples were collected. Fasting blood sugar (FBS), fasting serum insulin (FSI) and lipid profile were measured at the Pathology Department, Department of Medical Research. Plasma glucose level was determined by the glucose oxidase method and fasting serum insulin was measured by enzyme linked immunoassay (ELISA) kit method. DNA was purified from FTA card for PCR amplification. Polymerase chain reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) for IRS-1 gene was also done at Pathology Department, Department of Medical Research, Lower Myanmar, Yangon. HOMA IR and HOMA β-cell function were calculated using the formula by Matthews et al., in 1985.[9]

$$\text{HOMA IR} = \frac{\text{FPI}(\mu\text{IU/mL}) \times \text{FPG}(\text{mmol/L})}{22.5}$$

$$\text{HOMA } \beta\text{-cell function} = \frac{20 \times \text{FPI}(\mu\text{IU/mL})}{\text{FPG}(\text{mmol/L}) - 3.5}$$

Data were analysed using SPSS (version 16.0) statistical software. Overweight and obesity were defined according to WHO guideline (2006), overweight as BMI ≥25 kg/m^2, obese as BMI ≥30 kg/m^2. Data were presented as mean value ± standard deviation (SD). Comparison between two means was done using Student's *'t'* test (unpaired) and the difference was considered significant when the two-tailed p-value is <0.05. The differences in proportions of specific genotypes and alleles by BMI category (i.e., overweight or obese) were tested using Fisher's exact test or chi-squared test, as appropriate. The correlation between insulin resistance and triglyceride was determined using Pearson's correlation.

Ethical Consideration

The thesis including pro forma and written informed consent form was submitted to the ethical review committee to obtain ethical approval to conduct the research work. History taking, physical examination and 5 ml of blood sample were taken after getting informed consent. The study was approved by the Academic Board, University of Medicine 1, Yangon.

RESULTS

Among 100 overweight and obese patients with T2DM, 81 patients were of homozygous (G/G) genotype, 18 patients were of heterozygous (G/A) and only one patient of homozygous (A/A) genotype. The allele frequencies of "G" was 90% and that of "A" was 10% (Table 1). There was no statistically significant difference in the proportion of genotypes between overweight and obese T2DM patients.

Out of 100 patients, 70 were overweight (male: female=25:45) and 30 were obese (male: female =12:18). The mean age and BMI of the present study were 56.93±10.96 year and 28.35±4.36 kg/m^2.

The mean value of insulin resistance (HOMA-IR) calculated from FBS and FSI was 6.28±6.29 and β-cell function was 117.78±121.27 % in the present study.

There was no significant difference in FBS, FSI, HOMA-IR, β-cell function (Table 2), TC,TG, HDL and LDL between IRS-1 (G972R) carriers and non-carriers (Table 3).

Table 1. Genotype distributions and allele frequencies for G972R mutation in IRS-1 gene in patients with overweight and obese type – 2 diabetes mellitus

Variables	Obese	Overweight	Total (%)	Remark
Genotypes				
G/G	23	58	81 (81%)	
G/A	7	11	18 (18%)	p=0.583
A/A	–	1	1 (1%)	
n	30	70	100	
Allele				
G	53	127	180 (90%)	p=0.607
A	7	13	20 (10%)	
n	60	140	200	

Chi-squared test

Table 2. Fasting blood sugar, fasting serum insulin, HOMA-IR and β-cell function between IRS-1 (G972R) carrier and non-carrier

Parameters	Carrier (n=19)	Non-carrier (n=81)	Remark
Fasting blood sugar (mmol/L)	8.27 ± 2.10	8.24 ± 3.63	*p*=0.9725
Fasting serum insulin (μU/mL)	17.39 ± 11.34	17.47 ± 15.38	*p*=0.9830
(Log fasting serum insulin)	(1.15 ± 0.29)	(1.09 ± 0.34)	*p*= 0.4792
HOMA-IR	6.43 ± 4.63	6.25 ± 6.65	*p*=0.9114
(Log HOMA-IR)	(0.70 ± 0.31)	(0.62 ± 0.36)	*p*=0.3739
β-cell function(%)	84.90 ± 64.29	125.49 ± 130.2	*p*=0.1906
{Log β-cell function (%)}	(1.80 ± 0.34)	(1.83 ± 0.51)	*p*=0.8081

Table 3. Lipid parameters of IRS-1 (G972R) carrier and non-carrier

Lipid Parameters	Carrier (n=19)	Non-carrier (n=81)	Remark
Total cholesterol (mg/dL)	171.00 ± 27.21	164.43 ± 36.15	*p*=0.4592
Triglyceride (mg/dL)	186.84 ± 35.11	190 ± 38	*p*=0.7416
HDL (mg/dL)	37.94 ± 5.89	37.32 ± 8.12	*p*=0.7546
LDL (mg/dL)	95.84 ± 28.16	89.59 ± 37.75	*p*=0.4996

The mean level of TG and HDL in IRS-1 carrier group was 186.84±35.11 mg/dl, 37.94±5.89 mg/dl and that of IRS-1 non-carrier group was 190±38 mg/dl, 37.32±8.12 mg/dl, respectively. There was no significant difference between these two groups.

Figure 1 shows significant negative correlation between insulin resistance and TG level (r^2 = 0.0529, *p*=0.01).

DISCUSSION

The prevalence of IRS-1 (G972R) polymorphism was higher in obese patients with T2DM, and the prevalence of polymorphism is reported to be varied in various studies probably due to differences in genetics, race and ethnicity. The findings of the present study would highlight the genetic variation of polymorphism in T2DM in Myanmar and show whether the IRS-1 variant has effect on the insulin resistance, β-cell function and lipid parameters particularly in overweight and obese individuals.

In the present study, the G972R polymorphism was observed in 19% of T2DM. The prevalence of the G972R polymorphism appears to be higher than other Western (Danish, Finnish, African, Turkish and American) and Asian (Japanese, Taiwanese and Indian) studies.[7-13]

In the present study, out of 100 overweight and obese individuals, 18 patients were heterozygous (G/A) carriers and only one overweight patient was a homozygous (A/A) carrier. In Lei et al., and Yamada et al., the majority of the G972R polymorphism were of hetero-zygous (G/A) and there was no case of homozygous (A/A) carrier in patients with T2DM in Yamada's study.[14-16]

The insulin and glucose status, and the severity of diabetes mellitus were found to be higher in the population with higher G972R polymorphism prevalence. In the present study and also in the study of Lei et al., although the age and BMI were comparable, FBS and FSI levels were found to be higher. That might also apply to normal subjects because in the study of Yamada et al., it was reported that 2 people who were of homozygous G972R (A/A) substitution showed impaired glucose tolerance and a moderate degree of insulin resistance.[14-16]

The prevalence of G972R polymorphism does not seem to be related to BMI since the prevalence was quite high at 15.8% in Orkunoglusuer's study in which BMI was only 22.14±3.98 kg/m^2; yet it was only 4.2% in Ura's study with more or less similar BMI, 22.9±3.8 kg/m^2.[17,18]

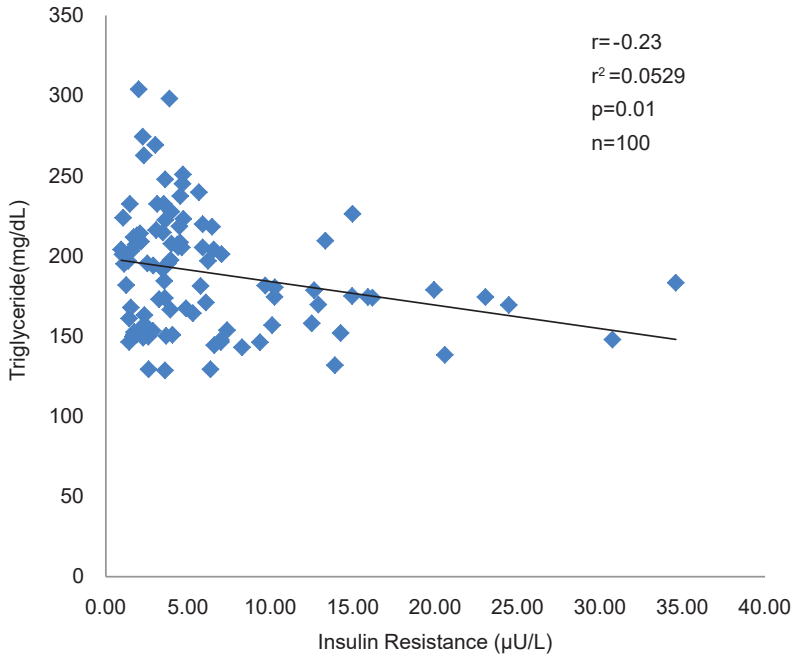

Figure 1. Negative correlation between insulin resistance and TG.
r = Pearson correlation coefficient; n = total numbers of subjects

In a meta-analysis by Jellema et al., no association between BMI and G972R was reported among individuals with BMI less than 27 kg/m^2.[5] However, in the study of Burguete-Garcia in 2010, a stronger association between the G972R and T2DM was reported among participants with BMI less than 23.1 kg/m^2 than among participants with BMI of at least 23.1 kg/m^2.[20]

The prevalence of G972R polymorphism in the present study was much higher than that reported in the Asian region. The BMI of other studies was found to be much lower than the present study {25.1±3.1 kg/m^2, 25.69±5.27 kg/m^2 versus 28.35±4.36 kg/m^2}.[19,21] However, the prevalence of G972R polymorphism in them was 1.1% and 1.8% respectively and that of the present study was many folds higher at 19%. Thus, it seems that factors other than regional and ethnic differences might have a role in the prevalence of polymorphism since the percentage of polymorphism differs between studies carried out in two places on the population with comparable BMI range.

The percentage of G972R polymorphism in the general population was 13% in the study of Imai et al., and 4% in that of Almind et al.[6,21] In the above studies, the prevalence of G972R polymorphism in T2DM was 23% and 11% respectively. In one combined analysis, it was reported that G972R substitution was present in 15% of 117 patients with T2DM and 7% of 94 normal subjects, indicating that the prevalence of G972R polymorphism was twice higher in persons with diabetes than in normal subjects. These observations are consistent with the hypothesis that mutations in the IRS-1(G972R) gene contribute to the pathogenesis of T2DM in 10-20% of the population.[21] Although the present study reported that G972R substitution was present in 19% of patients wuth T2DM, the study could not conclude G972R polymorphism was higher in persons with diabetes than the normal subjects because the present study did not determine G972R polymorphism in normal subjects.

However, no significant differences in FBS, FSI, β-cell function, and lipid parameters were observed between G972R carrier and non-carrier in the present study. It is thus suggested that G972R polymorphism alone may not impair the insulin, glucose and lipid status but other genetic, environmental and life style factors play a role in the development and progression of the disease. Therefore, analysis of polymorphism at sites other than 972 in IRS-1 gene and finding other genetic alterations and consideration of the risk factors seem to be required when any attempt is made to determine the role of genetic polymorphism in the disease pathophysiology.

The G972R polymorphism has 2 forms: heterozygous (G/A) and homozygous (A/A). Although it is not known whether genotypic difference has an effect on the insulin, glucose and lipid status, in the present study, only one homozygous case was found to be insulin resistant whereas 13 out of 18 heterozygous cases were insulin resistant. The allele frequencies of "G" was 90% and that of "A" was 10%. Neither the allelic frequency nor the genotypic frequency seems to be significantly different between the overweight and obese patients with T2DM, further disproving the notion of any relationship between BMI and gene polymorphism. Laukkanen et al., reported that among the common polymorphisms of the IGF-1R, IRS-1 and IRS-2 genes, IRS-1 and IRS-2 genes did not show the conversion from IGT to T2DM, whereas IGF-1R may regulate the risk of developing T2DM.[22] Moreover, Zhang and coworkers have analysed the same two polymorphisms in diabetic subjects participating in the United Kingdom Prospective Diabetes Study and found only an association between obesity and the β-3-adrenergic receptor polymorphism but not obesity and the IRS-1 polymorphism[23] but when both polymorphisms were present, there was a huge increase in the frequency of T2DM in Caucasian obese subjects.[24]

In the present study, HOMA-IR and fasting insulin were not correlated with total cholesterol, HDL, and LDL levels. However, significant negative correlation was observed between HOMA-IR and TG level. Although it was reported that TG level lowered after blood glucose control[25] in the group with high insulin resistance, such lowering of triglyceride level was also observed despite high fasting blood glucose. It is possible that in such cases with high HOMA-IR, hepatic TG synthesis is blunted and reduced. In addition, since insulin stimulates lipoprotein lipase activity which increases entry of free fatty acids for TG synthesis in the liver, insulin resistance would cause decreased TG synthesis.

Insulin leads to decreased gluconeogenesis and increased synthesis of fatty acids and TG in normal persons. Selective insulin resistance in the liver of mice with type 2 diabetes, insulin fails to decrease gluconeogenesis, but it continues to stimulate synthesis of fatty acids and TG. This produces the deadly combination of hyperglycemia and hypertriglyceridemia. Insulin fails to decrease gluconeogenesis, and it also fails to stimulate synthesis of fatty acids and TG in total insulin resistance in the liver of LIRKO mice. This leads to hyperglycemia without hypertriglyceridemia, a state that may have consequences less severe than those observed with the combined elevation.[26]

Zheng T et al.,[27] Zheng S et al.,[28] Ma M et al.,[29] reported that β-cell function was inversely related with TG level and TG promotes β-cell apoptosis. Therefore, lowering of TG level would reduce the β-cell inhibition effect by TG, and that seems to explain why in the present study fasting plasma insulin level was higher in the group with low TG level.

The present study reported that G972R substitution was present in 19% in patients with T2DM, the study could not conclude that G972R polymorphism was higher in persons with diabetes than the normal subjects because the present study did not determine G972R polymorphism in normal subjects. The present study determines only one genetic polymorphism in insulin signaling pathway and other genetic polymorphisms such as β -3-adrenergic receptor polymorphism could be considered to show correlation between genetic polymorphism and T2DM.

CONCLUSION

The prevalence of IRS-1 G972R polymorphism was 19% in overweight and obese T2DM patients in the present study. In this preliminary study, there are no differences in HOMA-IR, FBS, FSI and lipid parameters between the IRS-1 G972R carrier and non-carrier groups.

Insulin resistance was found in 72% and only 18% of the study group have good glucose control. Plasma TG level was higher and HDL level was lower than normal range and plasma TG level was significantly and inversely correlated with insulin resistance.

Triglyceride production falls at high insulin resistance in T2DM, suggesting that the fall in lipid parameters should not be taken only as good glycemic control. Measurement of FBS and FSI level is also recommended for formulating the efficient management strategies.

It was concluded that IRS-1 G972R polymorphism was not important in insulin resistance, β-cell function and lipid parameters in overweight and obese patients with T2DM. There could be a number of candidate genes in the pathophysiology of diabetes mellitus, genetic sequencing of IRS-1 and other genes in the insulin signaling pathway, and finding out the alteration in their genetic patterns would provide clues for the association of the site-specific polymorphisms of these genes with insulin resistance in T2DM.

Limitation of the study

The present study could not conclude whether G972R polymorphism was higher in overweight and obese T2DM patients than in normal weight subjects because the present study did not determine G972R polymorphism in normal subjects. The present study determined only one genetic polymorphism in the insulin signaling pathway and other genetic polymorphisms such as Human leucocyte antigen (HLA), glycogen synthase, glucagon receptor, β-3-adrenergic receptor polymorphism could be considered to show correlation between genetic polymorphism and T2DM.

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Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

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ORIGINAL ARTICLE

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An Exploration of Knowledge and Themes on Diabetes during Outpatient Consultation in a Tertiary Referral Hospital*

Angelique Bea Uy,[1] Harold Henrison Chiu,[1] Ramon Larrazabal Jr.,[2] Cecilia Jimeno[1]

[1]*Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of the Philippines College of Medicine-Philippine General Hospital*
[2]*Department of Medicine, University of the Philippines College of Medicine-Philippine General Hospital*

Abstract

Objectives. Effective communication has been correlated with improved outcomes in diabetes mellitus. Patient comprehension bears an effect on understanding, improving healthcare access and utilization, interaction with healthcare providers, caring for one's own health, and shared decision making. Currently, there is a gamut of information on diabetes-related terms from various sources. However, no material has yet been available for clinical use in the third world setting. Hence, we explored the most common themes discussed during an outpatient diabetes consult in our hospital.

Methodology. Consultation audio recordings (N = 96) and focus group discussions (N = 32) were conducted among adults with diabetes. Transcribed results underwent qualitative content and thematic analyses to develop the conceptual framework.

Results. The study generated the following themes: diabetes mellitus diagnosis, lifestyle modification, treatment targets, hypoglycemia precautions, diabetes complications, and medication safety. There was a good understanding of these themes among patients with a higher educational attainment, however, among those with lower educational attainment, the attitude of patients toward diabetes care is paternalistic.

Conclusion. The themes discussed in outpatient diabetes consult reflects the dimensions of diabetes care mainly influenced by socio-cultural factors, patient-doctor relationships and adaptability to limitations of resources. The results will be used to develop and validate a culturally appropriate diabetes health literacy tool.

Key words: diabetes mellitus, focused group discussion, health literacy

BACKGROUND

Diabetes mellitus is recognized as a challenge for many healthcare systems. It is a chronic disabling disorder which poses a burden not only for individuals with the disease but also to society. The deterioration of glucose control increases the incidence of diabetes-related macro- and micro-vascular complications such as blindness, stroke, cardiovascular events, chronic kidney disease and lower extremity amputations.[1]

Despite the advancement of scientific knowledge on the pathology and development of novel treatments in diabetes, the burden of the disease continues to escalate. A multidisciplinary approach is key to tackle the difficulties of chronic disease management to encourage effective self-care including diet and medication adherence, promoting physical activity and participation in preventive care strategies.[2-3]

Various factors have been identified as social determinants of health. Although there are many factors affecting the process of care and achieving improved health outcomes and quality of life in diabetes, provider linguistic and cultural competency has been seen as a knowledge gap needing to be addressed in culturally-specific diabetes management. Language barriers can impact the perception patients have of healthcare facilities. Simple advice ranging from what is the disease, the extent of the disease/complications, lifestyle modifications and medication adherence might be a problem if mistranslated.

Addressing language barriers caused by medical terminology may potentially curb health inequities.[4] Patient understanding is crucial to maximize participation in their own management to achieve adequate care and improve disease status; on the other side of the equation are the quality and capacities of the healthcare systems, organizations and professionals rendering care.[5-6] Health

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Corresponding author: Angelique Bea C. Uy, MD
Division of Endocrinology, Diabetes and Metabolism,
Department of Medicine, University of the Philippines
College of Medicine-Philippine General Hospital
Taft Avenue, Ermita 1000 Manila, Philippines
Tel. No.: +632-55484000
E-mail: acuy2@up.edu.ph
ORCiD: https://orcid.org/0000-0002-9539-8239

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professionals must establish and understand a patient's health literacy before delivering interventions or education.[7]

The Institute of Medicine describes an attribute of a health literate organization as having healthcare providers who use health literacy strategies during interpersonal communications with patients.[8] One significant challenge in the use of medical terminologies in health consults is the mismatch in functional and communicative aspect of the health literacy domain between the patient and the healthcare provider. A recent systematic review of health literacy sensitive diabetes interventions showed significant improvements in diabetes control with use of plain language, limiting teaching to 3 to 5 key points, and incorporating teach-back to ensure comprehension. However, healthcare providers have not consistently adopted these techniques.[9-11]

Currently, there is a gamut of information on diabetes terms to be found on the internet, in textbooks, handouts, etc. No material has yet been compiled based on the diabetes-related terms used in clinical practice in the third world and how these are defined in a culturally-appropriate perspective of both the healthcare provider and the patient.[12]

Effective communication has been correlated with improved outcomes. Patient comprehension during a medical consult has an effect on disease understanding; improving access and utilization of healthcare; interacting with healthcare providers; caring for one's own health; participating in health debates and decision making and greater satisfaction.[13] Doctors frequently introduce medical terms during consults that may not necessarily be highfalutin, but due to the lack of simpler terms are still used, impairing effective communication.[14-16] As such, it is important to identify commonly used diabetes medical themes during consult.

The aim of the study was to generate the most common themes discussed during an outpatient diabetes consult in a tertiary hospital, determine the level of knowledge and understanding of patients with diabetes of the most common diabetes medical terminologies utilized during consults, and to develop a conceptual framework determining the factors affecting diabetes care relating to the themes generated. This shall pave the way to better and effective communication of the previously known 'medical jargon' into scientifically meaningful terms because of better comprehension. This will lead to patient empowerment and decision-making with respect to self-care, medication adherence and complications prevention. In addition, the results of this study will also contribute to Filipino lexicography. Terminologies and definitions generated may be of use in constructing materials for more effective translation and expression of medical terms related to diabetes care.

METHODOLOGY

Our study was carried out in accordance with the principles outlined in the 2015 Declaration of Helsinki. It was approved by the Institutional Review Board and Research Ethics Board of the University of the Philippines Manila (UPM-REB Code: 2018-610, RGAO Registration No.: 2018-1166). Informed consent was obtained from the patients prior to the audio recordings and focused group discussions.

Study Design and Setting

This was a qualitative study consisting of transcription of patient consult recordings and focus group discussions conducted within the premises of the outpatient department of a tertiary hospital in the Philippines.

Study Procedures and Outcomes

Phase I. Diabetes Consultation Recordings and Transcription

The sample size for the audio recordings was 96 to represent the population of the physicians who conduct consultations in patients with diabetes. The recordings were stratified according to patient age (18 – 60 years vs. more than 60 years) and educational status (high school graduate or less vs. college or more). The patients were recruited in this phase of the study using purposive sampling. Recordings were undertaken once the patient has signified their informed consent during consults at the family medicine, internal medicine and endocrinology clinics, respectively (Table 1).

To generate a list of medical terms used during a diabetes consultation encounter, transcriptions of consultation audio recordings (with patient consent) and content analysis were performed by an independent observer. The diabetes-related medical terms were quantitatively tallied on how many times they were mentioned during consults, taking into consideration synonymous terms and grouping together of similar concepts before the final list of terms was generated.

Phase II. Focus Group Discussion (FGD)

To validate the list generated from Phase I, FGDs involving a group of diabetic healthcare experts and another group of adult diabetic patients selected via purposive sampling were done with the principal investigator as facilitator (Table 2).

Table 1. Summary of the number of audio recordings from the outpatient clinics stratified according to age and educational status

Clinic	Age range (years)	Educational status	Number of recordings
Family Medicine	18 – 60	High school graduate or less	18
	More than 60	College or more	18
Internal Medicine	18 – 60	High school graduate or less	18
	More than 60	College or more	18
Endocrinology	18 – 60	High school graduate or less	12
	More than 60	College or more	12

Table 2. Focus group discussion groupings stratified according to age and educational status

Group	Age range (years)	Educational status	Number of participants
1	18 – 60	High school graduate or less	8
2	More than 60		8
3	18 – 60	College or more	8
4	More than 60		8

The panel experts were as follows: 3 endocrinologists, 2 internal medicine physicians and 2 family medicine physicians. We did not include paramedical staff and social scientists in our study. The groups of adult patients with diabetes comprised of eight participants each, large enough to keep the conversation going, at the same time small enough to prevent people from being left out of the discussion;[17] each group was categorized according to age and educational attainment (Table 2).

The activity was conducted in a quiet room where the participants, including the facilitator, were seated face-to-face in a circle. It commenced with the facilitator first explaining the purpose and outline of the FGD. The following discussion points were tackled: 1) Identify the factors affecting health outcome in diabetes care; 2) Identify the role of diabetes consultation advice on diabetes control; 3) Identify medical terms commonly used during the consult; 4) Identify which medical terms are easily to poorly understood; and 5) Define in their own words each diabetes medical term.

Each participant was given a chance to speak during the entire duration of the activity. The facilitator noted both verbal responses and non-verbal cues including gestures and body language. The FGD concluded with the facilitator summarizing the key points of the discussion. The team reviewed the responses in order to validate the transcribed responses from the consultation recordings and the FGD responses.

Consensus was derived by the Delphi method, the standard technique for achieving convergence of opinion from a panel of experts. The following were the points for consensus: 1) Factors affecting health outcome in diabetes care; 2) Role of diabetes consultation advice on diabetes control; 3) Medical terms commonly used during the consult; 4) Medical terms that are difficult to understand; and 5) Own definition of the medical term diabetes. The Delphi method relies on a system of iteration and feedback to achieve a summation of comments, making each panel member aware of the range of opinions and the reasons underlying them. The method is also notable for its ability to provide anonymity, reducing the effects of group pressure for conformity, the presence of a controlled feedback process, and the suitability of a variety of statistical analysis techniques to interpret the data.[18]

The process took place over several rounds: In the first round, all panel experts were emailed a transcribed copy of the consultation recordings and FGD outputs. They were asked to individually place their opinions, as well as the underlying reasons for these. These responses were collected and summarized by a facilitator, who kept the identity of each panel expert anonymous. The generated diabetes medical terms commonly used during consultations were sent back to the individual panel experts for review, in light of the other panel members' replies.

This second round reveals areas of agreement and disagreement, and consensus begins forming.[19] Finally, during a third round (the minimum number recommended), the revised output was again sent back to the individual panel members, and the version approved by the majority (50% + 1, or at least four experts) was accepted.[20]

A conceptual framework was generated using the terms from the audio recordings, focus group discussion and consensus from the panel experts in order to summarize the important key factors that affect diabetes care during outpatient consults.

RESULTS

Phase I. Diabetes Consultation Recordings and Transcription

A total of 96 diabetes consults recordings were obtained from clinics in the Philippine General Hospital Outpatient department handling patients with diabetes: family medicine (FM) (n=36), internal medicine (IM) (n=36), and endocrinology (n=24). The patient groupings are summarized in Table 1. Diabetes consultation duration varied from 15 to 45 minutes in the FM Clinic, 2 to 49 minutes in the IM Clinic, and 3 to 35 minutes in the Endocrinology clinic.

The top ten most commonly used diabetes medical terminologies during a patient-doctor encounter in an outpatient clinic include: (1) Fasting Blood Sugar (FBS); (2) Hypoglycemia and its symptoms; (3) HbA1c; (4) Creatinine in relation to diabetic nephropathy; (5) Diet and Exercise; (6) BP Control; (7) DM Complications; (8) Symptoms of Diabetes Mellitus; (9) Treatment goals; and (10) Medication side effects. The most commonly repeated concept in each consult was diabetes complications. When re-examined, most complications were dominantly in loss-framed messages versus gain-framed messages in which the latter highlights the benefits of a specific behavior or a risk factor. These terms were validated by a method of triangulation through facilitated focus group discussions.

Phase II. Focus Group Discussion (FGD)

Focus group discussions were held to validate the top most commonly used diabetes-related terms elicited from the audio recordings of consults. Elicited in the FGDs were questions on factors affecting outcome on diabetes care. Factors identified were knowledge of the disease; understanding of the disease entity; reiteration of the healthcare provider of treatment plans; how to act upon the disease and treatment targets; and a stable patient-doctor

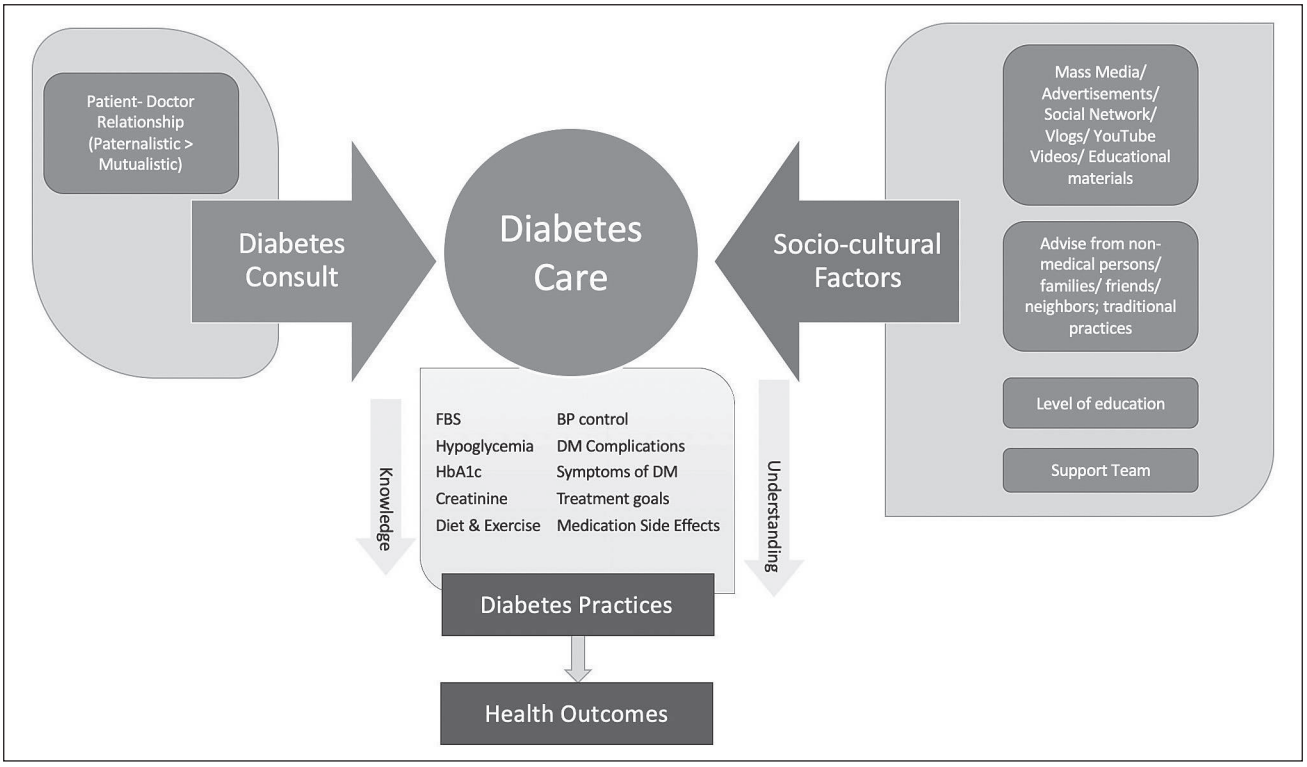

Figure 1. The conceptual framework illustrating the different identified factors that affect diabetes care during outpatient consults.

relationship guided by full trust of the doctor by the patient. It also showed a more paternalistic doctor-driven decision making versus a mutual patient-doctor relationship on the treatment plan especially on the choice of medications, the flexibility of the physician to change in treatment plans in cases of treatment failure and limitations of funds.

Phase III. Generation of Conceptual Framework

A conceptual framework (Figure 1) was then generated using the terms from the audio recordings, focus group discussion and consensus from the panel experts in order to summarize the important key factors that affect diabetes care during outpatient consults.

DISCUSSION

The Filipino language consists of a vast array of synonyms on how to express ideas. Language has the potential to benefit or hinder the attainment of proper health management. It was deemed worth further exploring each of the most common terms and themes generated during an outpatient diabetes consult to explain disease diagnosis, treatment, for patient counselling, prevention, and promote adherence to therapy.

Fasting blood sugar is a parameter used to diagnose diabetes mellitus. It is also used to monitor patients with diabetes for the appropriateness of sugar control. It is usually taken after a person has not eaten for eight to fourteen hours, usually overnight. Being the most commonly available and affordable screening and monitoring test for diabetes mellitus, it is not surprising that it is the most used diabetes-related term in a consult.

Hypoglycemia being a potentially dangerous state of low blood sugar usually less than 70 mg/dL, or *too little sugar in the blood* presents with signs including hunger, nervousness, shakiness, perspiration, dizziness or light-headedness, sleepiness, and confusion, is one of the symptoms most elicited by the physicians. Hypoglycemia is a diabetic emergency and a complication of treatment, and if persistent can lead to serious morbidities including but not limited to heart attacks, and even death. Patient-initiated Filipino terms pertaining to hypoglycemia includes symptoms such as *nanginginig, nangangatog, labis na pagkagutom, nahihilo, lupaypay, inaantok, pagkawala ng malay*. Noticeably, hypoglycemia treatment was not regularly advised in the Family Medicine and Internal Medicine clinics compared to the IM- Endocrine clinic. From the recorded consultation, around 70% from the IM-Endocrine recordings were insulin requiring, versus 40% in Family Medicine and Internal Medicine clinics. Simple culturally available measures to address hypoglycemia include consuming three pieces of hard candies, or a half-cup of juice or regular soft drinks; and to avoid skipping meals.

Hemoglobin A1c (HbA1c) is a test that measures a person's average blood sugar level over the past 2 to 3 months. Hemoglobin is the part of the red blood cell that carries oxygen to the cells and sometimes joins with the glucose in the bloodstream most especially in the setting of chronic hyperglycemia. Generally, this blood test is well understood by patients with diabetes with higher level of education i.e., some college or more, included in the study. This is their measure for control of blood sugars. It was elicited both from the individual audio recordings and the FGDs that there was a good understanding as to the purpose of this test.

Creatinine is another commonly inquired term during a diabetes consult. It is a surrogate marker for renal

dysfunction, as it is a waste product from protein in the diet and from the muscles of the body, and removed from the body by the kidneys. This term is simply known as *crea* to the patients with diabetes. The association that as the creatinine increases, kidney function decreases is widely understood by our patients. Diabetes nephropathy is equated to *kidney damage, kidney failure,* and *dialysis.*

Lifestyle modifications through revision of diet and institution of exercise have also been reiterated during consults. Queries have focused primarily on the proper nutrition composition and the types of exercises best suited for patients with diabetes. The usual advice given by physicians include adapting the *pinggang pinoy*, a low-fat, low-calorie diet and moderate exercise usually brisk walking for at least 30 minutes, 5 days a week. Diabetes meal planning options for meal substitutions for different food groups are given to patients, however, the details of such interventions are often left to the nutritionist for further discussion. Referrals to the dietary service are done after each consult most commonly observed from the IM-Endocrine clinic.

Blood pressure, simply known as *BP* or *presyon* to patients control is emphasized in diabetes management. It has been shown that a good BP control of less than 140/90 is targeted for patients with diabetes for slowing down kidney damage and minimizing albuminuria. The initiation of ACE-inhibitors or ARBs are given as options to patient to achieve this. However, this is not prioritized by patients with diabetes included in the study because of the lack of knowledge on the effects of blood pressure control on kidney function and deterioration.

Diabetes mellitus complications included in consults aside from nephropathy, are neuropathy including bilateral loss of sensation in distal extremities specifically the hands and the feet described as *pamamanhid, pangangalay ng mga kamay at paa*, *matagal na paghilom ng sugat, pagkaputol ng paa,* erectile dysfunction, constipation, delayed digestion manifesting as nausea, vomiting or bloating *pagkabilis mabusog*, autonomic neuropathy presenting as orthostatic dizziness *pagkahilo lalo na kapag nagbago ng posisyon*; retinopathy presenting as loss of vision *panlalabo ng mata, pagkabulag*; and macrovascular complications including heart attack, heart failure, stroke, and amputations.

Symptoms of Diabetes Mellitus elicited are polyuria, nocturia, increased thirst, unintentional weight loss, and paresthesia. These terms are translated as *madalas na pagihi, labis na pagka-uhaw, pamamayat o pagbabawas ng timbang*, and *pamamanhid ng mga kamay at paa*.

Treatment goals for patients with diabetes are inquired by patients and discussed by physicians. Different targets of HbA1c, fasting blood sugar, BP, lipid level, and weight loss are set depending on age, and the presence of co-morbidities. Generally, for younger adults with diabetes without CV risk an A1c of 6.5-7.0% is targeted, and if older with co-morbidities, a more relaxed target is recommended. This is individualized and explained to each patient.

Medication side-effects are often a cause of worry for patients with diabetes. More so, the bulk of the information is published on social media, websites, newspapers, and advice from neighbors about unvalidated effects of some medications, i.e., metformin causing cancer or renal failure, diabetes medications causing weakness, use of insulin as a 'death sentence' in a patient's diabetes state. Physicians should additionally educate patients regarding medication side-effects and efficacy of diabetes control during consults. Furthermore, the use of non-FDA approved food supplements with no therapeutic value were also brought up. Common lay terms describing side-effects include *pananakit ng tyan, pagsusuka, labis na pagbaba ng lebel ng asukal sa dugo, pananaba.*

The diabetes consultation has a key role in filling in the knowledge gap about the disease, and its consequences on the health outcomes of each patient. However, the patients are admittedly easily lured by mass media-advertisements/ TV commercials, YouTube videos, advice from colleagues, friends and neighbors, and social media releases by the non-experts.

Although it occurred in the top ten most commonly used terms in diabetes consults, the most poorly understood concept is that of the medication efficacy and side-effects. This serves as a limitation to achieving adherence to medication, good glycemic control, and better health outcomes.

The FGD group with a lower educational background is more or less familiar with the diabetes-related consultation terms, however, they are more reliant on its implications, and treatment adjustments to their physicians, rather than understanding the totality and course of their diabetes management. How this will affect treatment outcomes is yet to be determined in future studies.

The themes we have elicited in our study will allow us to develop a culturally-appropriate diabetes health literacy tool. The use of this tool will allow us, diabetes healthcare providers, facilitate better and more effective communication resulting in better patient comprehension with regards to self-care, medication adherence and complication prevention, and further to develop a diabetes education approach that is standardized and fit in our healthcare system. As some studies have shown, some patients do not think of a diabetes consult, including diabetes education a necessity because they do not consider themselves ill. However, we have to help patients realize that knowing the course of the disease and diabetes management can influence their health outcomes.

CONCLUSION

In summary, the themes discussed in outpatient diabetes consults reflects the dimensions of diabetes care, being influenced mainly by socio-cultural factors, patient's having a good grasp of the disease process, a trusting patient-doctor relationship and adaptability to limited resources. The study results will be used to develop and validate a culturally appropriate diabetes health literacy tool.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

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ORIGINAL ARTICLE

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History of Severe Hypoglycemia in Type 2 Diabetes Mellitus Unmasked Significant Atherosclerotic Coronary Artery Disease: A Comparative Case Control Study

Mohd Asyiq Raffali,[1] Syawal Faizal Muhammad,[1] Hamzaini Abdul Hamid,[2] Azmawati Mohammed Nawi,[3] Nor Azmi Kamaruddin[1]

[1]*Department of Medicine, UKM Medical Centre, Kuala Lumpur, Malaysia*
[2]*Department of Radiology, UKM Medical Centre, Kuala Lumpur, Malaysia*
[3]*Department of Community Health, UKM Medical Centre, Kuala Lumpur, Malaysia*

Abstract

Objectives. A history of severe hypoglycemia (SH) is associated with cardiovascular (CV) events among patients with type 2 diabetes mellitus (T2DM). In this study, we compared the severity of atherosclerotic coronary artery disease (ACAD) in T2DM patients with and without a history of SH.

Methodology. We conducted a comparative case-control study involving 28 T2DM patients with a history of SH within the last 5 years with no documented ACAD, and matched them with 28 T2DM patients with no history of SH. All subjects underwent coronary artery calcium scoring (CACS) with or without coronary computed tomographic angiography (CCTA) to evaluate the severity of ACAD.

Results. A history of SH in T2DM was associated with a higher prevalence of significant ACAD (79% versus 46%, *p*=0.026). A high CACS (≥100) was seen in a greater number of patients with a history of SH compared to those without (75% versus 43%, *p*=0.029). Similarly, there was a higher prevalence of obstructive CAD in those with a history of SH compared to those without (72% versus 39%, *p*=0.036). Median C-reactive protein level was also higher among patients with a history of SH (0.41 mg/dL versus 0.16 mg/dL, *p*=0.029).

Conclusion. In patients with T2DM, a history of SH is significantly associated with ACAD compared to those without SH. A history of SH warrants screening for ACAD.

Key words: hypoglycemia, coronary artery disease, type 2 diabetes mellitus, C-reactive protein

INTRODUCTION

Hypoglycemia is a common adverse complication of intensive glycemic control in several T2DM prospective studies. When a patient requires third party intervention to rectify hypoglycemia, the event is called severe hypoglycemia (SH). SH has been identified to be one of the strongest predictors of cardiovascular events, adverse clinical outcomes and mortality in people with T2DM.[1-4]

Hypoglycemia affects the cardiovascular system via augmented sympatho-adrenal responses. These cause electrophysiologic derangements which precipitate arrhythmias, an increase in cardiac workload leading to potential reduction of myocardial perfusion, and a tendency to induce a prothrombotic state and systemic release of numerous inflammatory markers.[5-9] These physiological responses to hypoglycemia may be harmful for people with a long history of T2DM, as they may have already developed significant atherosclerotic coronary heart disease, autonomic dysfunction and underlying cardiomyopathies.

Most T2DM patients who developed episodes of SH are treated as having isolated events due to consequences of therapy, inadequate caloric intake or strenuous exercise. They are only subjected to extensive atherosclerotic coronary artery disease (ACAD) screening when they develop symptoms suggestive of CAD or are admitted for acute coronary syndrome.

We sought to compare the prevalence of ACAD in patients with T2DM with and without a history of SH. To our knowledge, there are no published studies that have objectively determined the severity of ACAD in T2DM patients with a history of SH and its relationship with inflammatory biomarkers. Our findings may help aid us in understanding SH in T2DM patients as an indicator of significant ACAD.

METHODOLOGY

Research design, setting and participants

This is a comparative case-control study conducted in our institution from December 2019 to July 2020. We recruited

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Corresponding author: Mohd Asyiq Raffali, MD
Department of Medicine, UKM Medical Center
Jalan Yaacob Latif, Bandar Tun Razak 56000
Kuala Lumpur, Malaysia
E-mail: ashkashick@yahoo.com
ORCiD: https://orcid.org/0000-0003-0797-3342

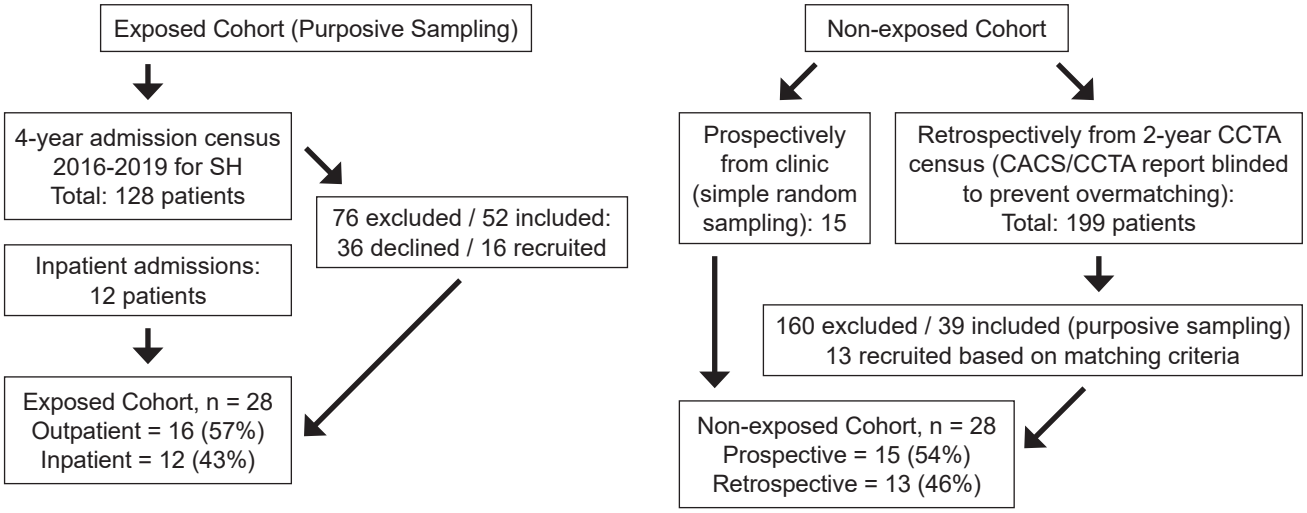

Figure 1. Patient recruitment flow chart.

28 T2DM patients with a history of SH within the last five years and closely matched them with 28 T2DM patients with no history of SH.

The inclusion criteria for our cohort with a history of SH were T2DM, with a history of glucometer-documented SH within the last five years. We excluded patients with the following characteristics: type 1 diabetes mellitus; age under 40 years; creatinine clearance less than 30 mL/min/1.73 m^2; established ACAD before index CT scan; and established risk of developing SH, such as advanced malignancy, advance chronic liver disease, adrenal insufficiency and deliberate overdose on oral hypoglycemic agents or insulin.

Our matching criteria for both cohorts were calculated based on propensity score matching on characteristics such as age, gender, ethnicity, duration of diabetes, smoking status, comorbidities, functional status, body mass index, creatinine clearance and type of medications.

For the definition of SH in our study, we did not set a minimum capillary glucose level, as each patient varies in his or her threshold for developing symptoms of hypoglycemia. We included any episode of symptomatic hypoglycemia that required third party intervention, such as administration of oral or intravenous glucose or administration of glucagon, with resolution of hypoglycemic symptoms after correction. Recurrent SH was defined as more than one episode of SH in a span of at least a month.

To reduce the probability of misclassification bias for any history of SH, we recruited patients with a diagnosis of SH and glucometer-documented reading during admission from our discharge registry. To address misclassification bias to ensure the absence of baseline ACAD, medical records were reviewed to determine documented history of ACAD rather than relying on recall. As the number of patients with a history of SH (exposed cohort) was scarce, purposive sampling was done to accomplish the minimal sample size. For the cohort without a history of SH (non-exposed cohort), prospective patients were sampled with simple random sampling after the exposed cohort were recruited. Retrospective patients were sampled based on matching criteria with the blinded CACS/CCTA report (Figure 1).

During recruitment of the exposed cohort from our hospital database, 52 out of 128 patients met the inclusion criteria. Sixteen patients agreed to volunteer for the study; the other 36 patients refused to be recruited or were uncontactable. As our study incorporated CT scan and intravenous contrast administration, most were reluctant to proceed. To attain the minimum number of patients, we also recruited inpatients who had episodes of severe hypoglycemia who required interventions.

Our study outcomes were based on three main parameters. For the first parameter, clinical CVD risk scoring, we employed the widely used American College of Cardiology/American Heart Association (ACC/AHA) (2013) and Framingham (2008) CVD risk scores. Both risk scores predict the risk of CVD within the next 10 years.[10,11]

For the second parameter, levels of inflammatory biomarkers, we used high-sensitivity C-reactive protein (hs-CRP) as it is well known to prognosticate CVD risk based on multiple epidemiological and interventional studies.[12,13] We also used serum matrix metalloproteinase-9 (MMP-9) to demonstrate the degree of arterial inflammation and risk of plaque destabilization and rupture. In coronary atherosclerosis, there is an enhanced expression of this MMP which is predictive of the severity of disease.[14-17]

For the third parameter, severity of ACAD, we used coronary artery calcium scoring and coronary computed tomography angiography, as many studies indicate that a negative CCTA and CACS of zero can effectively rule out obstructive CAD. In a 2008 meta-analysis, 64-slice CCTA had a sensitivity of 99% and negative predictive value of 100% for patient-based detection of significant CAD. As all our study patients were asymptomatic, a non-invasive test was a more acceptable modality for the diagnosis of ACAD.[18-22]

Selection bias was minimized via purposive sampling of our exposed cohort based on our inclusion and exclusion

criteria. Patient selection for the non-exposed cohort was performed using simple random sampling from the outpatient clinics.

Ethics statement

This study protocol was reviewed and approved by our institutional review board for ethics (Internal Review Board Reference Number: FF-2019-391). Written consent was obtained from all patients involved in the study prior to their participation. After proper counseling, all patients understood the risks and benefits of their involvement in the study.

Sample size calculation

We utilized the Kelsey formula with two-sided significance level of 95%, power of 80% and 1:1 participant ratio for both groups in our sample size calculation. As there are no studies in literature on the prevalence of ACAD in persons with T2DM with a history of SH, we set 80% as the expected prevalence of significant CAD in T2DM patients with history of SH, and 40% as the expected prevalence of significant CAD in the general population of T2DM patients without a history of SH.[23] The minimum sample size calculated was 26 subjects. We recruited 28 patients each in the case and control arms, to compensate for any study dropouts.

Laboratory measurements

Laboratory blood biochemistry measurements were done at the Department of Chemical Pathology of our institution. Tests included full blood count, renal profile, fasting lipid profile, hemoglobin A1c (HbA1c) and hs-CRP. For serum MMP-9 levels, we used the MMP-9 ELISA test kit (BioLegend®, United States of America) and performed duplicate tests at our research laboratory. To reduce the confounding effects of acute inflammation on hs-CRP or MMP-9 levels, we performed venesection at least 2 weeks from any history of febrile episodes.

Computed tomography scan protocol

For our CACS and CCTA protocol, we used a single fast-gated 640-slice helical CT (Toshiba Aquilion ONE™, Japan). A non-contrast-enhanced, prospectively ECG-triggered CT was performed initially to calculate the CACS using the Agatston method. CCTA was then performed with an intravenous injection of a bolus (80 to 100 mL at 4 to 6 mL/s) of non-ionic iodinated contrast agent (iopromide 370 mg/mL, Ultravist™, Bayer Healthcare, Germany) followed by a saline chaser (50 mL at 4 to 6 mL/s). If the heart rate was monitored to be >65 beats per minute, beta-blockers (oral metoprolol 50 to 100 mg) were provided if tolerated. Sublingual nitroglycerin (0.5 mg) was also administered before the examination to optimize visualization of small coronary vessels.

For patients with extremely high CACS (≥800 Agatston units), the interpretation of the CCTA images will be suboptimal due to the high degree of calcification obscuring the true lumen of the vessel, thus reducing the specificity for detection of ACAD. Most of these patients were excluded from proceeding with CCTA to avoid the possibility of false negative results.[24] Comparison of severity of ACAD based on CCTA was only done in patients who completed CCTA investigation.

Computed tomography scan data analysis

An overall CACS was documented for each patient based on the scoring algorithm of Agatston et al., where coronary artery calcium was identified as a dense area greater than 1 mm^2 in the coronary artery exceeding the threshold of 130 Hounsfield units.[25] A CACS level of ≥400 was chosen as the cutoff level of significant ACAD.[22,26] All CCTA investigations were evaluated by two experienced observers, using a standard approach of analysis.

We created two simple CAD severity scoring methods based on the CCTA for this study. The first describes the percentage of coronary artery segments involved, that is, the percentage of coronary artery segments with plaques regardless of degree of stenosis (e.g., 0% indicating no plaque involvement in all segments and 100% indicating all segments were involved with plaque). The second describes the percentage of severity of segment stenosis, calculated as the percentage of total sum of scores of all segments based on severity of stenosis to maximum possible score (e.g. 0 indicating no plaque, 1 with plaque present or mild stenosis at <50%, 2 with moderate stenosis of 50 to 75%, and 3 with severe stenosis of >75%).

Statistical analysis

Categorical variables were presented as frequency rates and percentages. Continuous variables were depicted as mean [standard deviation (SD)] if they were normally distributed and median [interquartile range (IQR)] for non-normally distributed data. The means for continuous variables were compared using independent group t-tests in normally distributed data. Otherwise, the Mann-Whitney U test was used. Proportions for categorical variables were compared using the χ^2 test, although the Fisher exact test was used when data were limited. All statistical analyses were performed with SPSS® (version 25.0) (IBM®) for Windows. A 2-sided $p<0.05$ was deemed statistically significant.

RESULTS

Baseline characteristics

Despite matching baseline characteristics, patients with a history of SH had higher median ACC/AHA CVD and Framingham risk scores of more than 30%, but the difference did not reach statistical significance.

For laboratory investigation, T2DM patients with a history of SH had higher levels of total cholesterol and low density lipoprotein-cholesterol (LDL-C), but lower levels of high density lipoprotein-cholesterol (HDL-C) and HbA1c levels. This might account for the higher clinical risk scores and susceptibility to SH. However, all the differences failed to reach statistical significance.

The hs-CRP levels were significantly higher among patients with a history of SH, compared to those without (p=0.029). This finding suggests that patients with a history of SH had a higher degree of pro-inflammatory state, with subsequent greater risk of plaque rupture and destabilization (Table 1).

Comparison of CCTA and CACS

We performed CACS for all patients. However, 12 (21%) patients (10 from the SH group and 2 from non-SH group) did not proceed with CCTA mostly due to extremely high

Table 1. Baseline characteristics

Characteristics	Severe Hypoglycemia		*p* value
	Yes (n=28)	No (n=28)	
Demographic			
Female gender (%)	15 (54)	15 (54)	1.000
Age†, year (SD[a])	65.35 (8.99)	64.54 (8.74)	0.730
Background and Comorbidities			
Hypertension	24 (86)	25 (89)	1.000
Hyperlipidemia	25 (89)	27 (96)	0.611
Obese (body mass index ≥30 kg/m^2)	2 (7)	5 (18)	0.422
Active smoking	7 (25)	8 (29)	1.000
History of CVA[b]	4 (14)	4 (14)	1.000
Clinical Parameters			
Body mass index†, kg/m^2 (SD[a])	24.99 (3.56)	25.14 (4.91)	0.893
Systolic blood pressure†, mmHg (SD[a])	130.82 (11.20)	130.21 (11.57)	0.843
Duration of diabetes‡, years (IQR[c])	10 (6.25-20.00)	10 (6.50-14.75)	0.987
On a sulphonylurea	9 (32)	9 (32)	1.000
On insulin injection	9 (32)	8 (29)	1.000
Framingham (2008) Score >30%	18 (64)	15 (54)	0.587
ACC/AHA CVD Risk Score[d]‡, (IQR[c])	25.90 (14.25-35.95)	22.40 (8.88-31.83)	0.461
Laboratory Results			
HbA1c[e]‡, % (IQR[c])	6.85 (6.25-9.73)	7.90 (6.65-9.10)	0.333
Creatinine clearance†, mL/min/1.73 m^2 (SD[a])	72.91 (19.88)	71.68 (22.92)	0.831
Total cholesterol†, mmol/L (SD[a])	4.64 (1.23)	4.49 (1.34)	0.656
LDL-C[f]‡, mmol/L (IQR[c])	2.54 (1.96-3.38)	2.51 (1.83-2.93)	0.491
HDL-C[g]†, mmol/L (SD[a])	1.20 (0.40)	1.31 (0.37)	0.301
hs-CRP[h]‡, mg/dL (IQR[c])	0.41 (0.19-1.74)	0.16 (0.06-0.62)	0.029
MMP-9[i]‡, pg/mL (IQR[c])	4394.00 (3049.75-14081.25)	4117.00 (2648.50-7449.75)	0.235

† Mean value
‡ Median value
[a] SD, standard deviation
[b] CVA, cerebrovascular accident
[c] IQR, interquartile range
[d] ACC/AHA CVD Risk Score, American College of Cardiology/American Heart Association cardiovascular disease risk score
[e] HbA1c, hemoglobin A1c
[f] LDL-C, low density lipoprotein-cholesterol
[g] HDL-C, high density lipoprotein-cholesterol
[h] hs-CRP, high-sensitivity C-reactive protein
[i] MMP-9, matrix metalloproteinase-9

CACS (≥800), known to affect the objective interpretation of the CCTA; technical issues such as suboptimal images; or uncontrolled heart rate. Median CACS was significantly higher in the SH group compared to the non-SH group. For CACS subgroup analysis, the SH group had more patients with CACS ≥100.

In terms of CCTA features, there were only a few patients in the SH group with no significant stenosis. Obstructive CAD in each of the epicardial coronary arteries was found to be more prevalent in the SH group, with a statistically significant finding for the right coronary artery. Both scores that we created to compare the severity of coronary artery disease were also higher in the SH group: the mean percentage of segments involved and the mean percentage of segment severity were comparatively greater than in the non-SH group, but the difference did not reach statistical significance.

The SH group had more patients with significant CAD, based on CACS ≥400 and/or presence of at least one epicardial coronary artery stenosis ≥50% (OR 4.231, 95% CI: 1.314 to 13.617, *p*=0.026). The 46% prevalence of significant ACAD in the non-SH T2DM patients correlates well with previous studies which employed CCTA for detection of significant CAD (Table 2).[27,28]

Multinomial logistic regression was done to determine the relationship of SH and other confounders for significant ACAD, such as age, gender, smoking status, family history of premature CVD, creatinine clearance, body mass index, HbA1c level, HDL-C, LDL-C, Framingham risk score and ACC/AHA risk score. Only a history of SH was determined to be statistically significant as an independent risk factor for ACAD (*p*=0.004).

Subgroup analysis within the SH group

A. Recurrent SH versus single episode of SH

There were 5 (18%) patients with recurrent episodes of SH. Compared to patients with a single episode of SH, those with recurrent SH were older and had lower creatinine clearance. These may explain the higher risk of recurrent severe hypoglycemia. All the patients with recurrent SH had significant CAD. The hs-CRP and MMP-9 levels in these patients were also higher, suggesting a greater risk for CV events, but the differences from patients with single SH did not reach statistical significance. CACS were higher in patients with recurrent SH, with a difference that was statistically significant. However, because most patients had extremely high CACS (≥800), CCTA was not performed in this group (Table 3A).

B. Onset of SH within the first month versus more than one month

There were 10 (36%) patients who were investigated within the first month of the occurrence of SH. The hs-CRP was higher in this group compared to those

Table 2. Comparison of computed tomographic calcium score and angiography between groups

Characteristics	Severe Hypoglycemia		*p* value
	Yes (n=28)	No (n=28)	
Coronary artery calcium score‡, (IQR[a])	277 (80-869)	56.50 (14.25-458.75)	0.030
0	2 (7)	3 (11)	1.000
1-99	5 (18)	13 (46)	0.044
100-399	9 (32)	5 (18)	0.355
100 or more	21 (75)	12 (43)	0.029
400 or more	12 (43)	7 (25)	0.259
Coronary CT[b] angiography			
Completed coronary CT[b] angiography (%)	18 (64)	26 (93)	NA
Segment involvement percentage†*, % (SD[c])	39.35 (30.36)	33.12 (28.12)	0.488
Segment severity percentage†**, % (SD[c])	20.49 (18.35)	14.88 (13.47)	0.248
No significant stenosis	5 (28)	16 (62)	0.036
Single vessel disease	6 (33)	3 (12)	0.128
Two vessel disease	2 (11)	4 (15)	1.000
Three vessel disease	5 (28)	2 (8)	0.103
Stenosis ≥50% involvement			
Left main stem	2 (11)	0	0.162
Left anterior descending (LAD) (including diagonal 1)	11 (61)	9 (35)	0.125
Left circumflex (LCx) (including obtuse marginal)	6 (33)	5 (19)	0.314
Right coronary artery (RCA)	8 (44)	4 (15)	0.045
Results			
At least one coronary artery with ≥50% stenosis	13 (72)	10 (39)	0.036
Significant CAD[d] (CACS[e] ≥400 and/or at least one major epicardial coronary artery disease ≥50% stenosis)	22 (79)	13 (46)	0.026

† Mean value
‡ Median value
* Segment involvement percentage is the percentage of segments with plaques regardless of degree of stenosis
** Segment severity percentage is the percentage of total sum of scores of all segments based on severity of stenosis: 0 = no plaque, 1 = plaque present or mild stenosis <50%, 2 = moderate stenosis 50-75%, 3 = severe stenosis >75%
[a] IQR, interquartile range
[b] CT, computed tomography
[c] SD, standard deviation
[d] CAD, coronary artery disease
[e] CACS, coronary artery calcium score

Table 3A. Comparison of characteristics of patients with recurrent or single episode of severe hypoglycemia

Characteristics	Recurrent (n=5)	Single (n=23)	*p* value
Age†, year (SD[a])	68 (61-78)	64.78 (48-84)	0.405
CKD G3[b] (%)	3 (60)	5 (22)	0.123
On insulin (%)	3 (60)	6 (26)	0.290
Creatinine clearance†, mL/min/1.73 m^2 (SD[a])	57.47 (18.20)	76.26 18.94)	0.083
hs-CRP[c]‡, mg/dL, (IQR[d])	0.45 (0.16-12.64)	0.39 (0.19-1.95)	0.696
MMP-9[e]‡, pg/mL, (IQR[d])	10770.00 (6800.50-16338.50)	3607.00 (2797.00-9896.00)	0.087
CACS[f]‡ (IQR[d])	668.00 (448.50-4851.50)	202.00 (65.00-677.00)	0.044

† Mean value
‡ Median value
[a] SD, standard deviation
[b] CKD G3, chronic kidney disease glomerular filtration rate category 3
[c] hs-CRP, high-sensitivity C-reactive protein
[d] IQR, interquartile range
[e] MMP-9, matrix metalloproteinase-9
[f] CACS, coronary artery calcium score

Table 3B. Comparison of characteristics of patients investigated within the first month or beyond the first month of severe hypoglycemia

Characteristics	Within first month (n=10)	Beyond first month (n=18)	*p* value
Age†, year (SD[a])	60.90 (6.89)	67.83 (9.41)	0.048
hs-CRP[b]‡, mg/dL, (IQR[c])	2.05 (0.25-8.69)	0.38 (0.12-0.49)	0.045
MMP-9[d]‡, pg/mL, (IQR[c])	7269.50 (3414.25-16226.00)	3444.50 (2853.50-10178.25)	0.308

† Mean value
‡ Median value
[a] SD, standard deviation
[b] hs-CRP, high-sensitivity C-reactive protein
[c] IQR, interquartile range
[d] MMP-9, matrix metalloproteinase-9

who had SH more than a month earlier, signifying a possible temporal association of SH with the process of inflammation and risk of plaque rupture (Table 3B).

DISCUSSION

In our cohort of patients with T2DM who had a history of SH, we found higher hs-CRP and more severe coronary artery disease based on imaging compared to those without any history of SH. The odds of having significant ACAD in patients with a history of SH based on this study was four-fold compared with other T2DM patients with no history of SH with matched baseline characteristics (OR 4.231, 95% CI: 1.314 to 13.617, p=0.026).

The relationship between SH and CV events and mortality are supported by analyses of randomized clinical trials, cohort studies and meta-analyses. The adjusted hazard ratios for total mortality of patients experiencing at least one episode of SH in comparison to those with no SH in large prospective randomized trials have been shown to be between 1.67 and 4.28.[29] What is missing in these data is the extent of the severity of ACAD in these patients that contributed to the higher risk of mortality.

To our knowledge, there are no published studies that have sought to objectively compare the severity of ACAD in T2DM patients who had a history of SH compared to those who never had SH. Our study had excluded patients with advanced renal impairment (creatinine clearance less than 30 mL/min/1.73 m^2) and matched them according to multiple CVD risk factors to determine whether a history of SH was an independent risk factor for severe ACAD.

SH has been known to promote an atherogenic state by hypersecretion of catecholamines and pro-inflammatory cytokines, leading to platelet aggregation.[30] In the acute state, hypoglycemia increases susceptibility of the myocardium to post-ischemic reperfusion injury and hampers the patient's ability for ischemic preconditioning.[31] The long-term cardiovascular effects of repeated hypoglycemia are due to increased endothelial dysfunction and a pro-inflammatory state, which contribute further to atherosclerosis.

Higher hs-CRP values were also seen in patients with SH, suggesting a more atherogenic and pro-inflammatory state. Serum MMP-9 levels, a marker of potential plaque destabilization and risk of rupture in ACAD, were also higher in this population, but this did not reach statistical significance. In addition, we demonstrated an apparent dose-response effect of SH, as seen in the significantly higher CACS in patients with recurrent SH compared to those with a single episode of SH.

Another significant finding is the temporal association between the onset of SH and elevated hs-CRP which demonstrates a higher risk for CV events. In this study, hs-CRP levels were significantly higher in the group with SH investigated within the first month of developing SH. Previous studies had shown that risk for CV events were higher within the first year of onset of SH, as compared with later years.

There have been doubts raised regarding the precise pathophysiological link between SH and CVD. Two large randomized control trials, DEVOTE 3 and TECOS, did not show a significant result between a history of SH and subsequent CV events.[32,33] Due to inconsistent results, SH was only perceived as a risk factor for CVD rather than a direct cause of CVD, as SH arguably occurs mostly in patients with advanced diabetes, advanced renal disease, on multiple oral anti-diabetes agents or high doses of insulin. Patients with these characteristics already have a higher risk for CVD to begin with.

The establishment of direct causality link between severe hypoglycemia and CVD will be difficult, as a prospective intervention study to compare a group with SH and without SH is needed. However, a large retrospective analysis done in Korea that assessed causality of SH with CVD by looking at strength, temporality, dose-response, consistency and biological plausibility of the relationship found all these factors to be significant.[34]

The recent Malaysian guidelines on the primary and prevention of CVD suggest adjustment of anti-diabetic medications to reduce the risk of hypoglycemia. A less stringent approach to glycemic targets will also be needed. Their overall CV risk profile should be reassessed and optimized to reduce the risk of CVD.[35] On the issue of screening asymptomatic patients for ACAD based on prior SH alone, there is no randomized clinical trial to determine if screening of CVD is beneficial. We recommend an individualized approach based on a premise of strict CVD risk stratification.

Numerous studies have suggested that screening asymptomatic patients with diabetes for CAD confers no additional benefit to the final outcome, even in patients with confirmed subclinical CAD.[36-38] Most patients who have experienced SH already have multiple risk factors for CAD, such as advanced age and chronic kidney disease.[39]

Limitations and Strengths

We identified a few limitations in the study. Our study was performed in a single center and was limited to a specific geographical area. Large-scale studies involving multiple centers in other areas of the world are needed to validate our results. Our definition of significant ACAD is also based on anatomical assessment of coronary artery disease. Combining anatomic with functional assessment such as myocardial perfusion imaging may provide a more prognostic value.

The strength of this study was that our exposed cohort was mainly based on patients with capillary blood glucose-documented episodes of SH, and not based on any unsubstantiated claim that led to misclassification of SH. We also compared our exposed cohort with controls of matched risk factors, thus reducing confounding factors affecting severity of ACAD. For objective assessment of ACAD, we employed a rather accurate and reproducible imaging technique by means of CCTA and CACS.

CONCLUSION

The results of the study suggest the prognostic importance of a prior history of SH, biological plausibility, dose

response and temporal association on the severity of ACAD. In patients who experienced SH despite not having typical CAD symptoms, timely cardiac assessment will be vital to prevent future major cardiovascular outcomes. A larger and more objective study perhaps is needed to discern the direct causality of SH on the severity of CAD.

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

Author Disclosure
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

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Baseline Glycemic Status and Outcome of Persons with Type 2 Diabetes with COVID-19 Infections: A Single-Center Retrospective Study

Marion Sarigumba, Jimmy Aragon, Ma. Princess Kanapi

Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, Makati Medical Center, Makati City, Philippines

Abstract

Introduction. The coexistence of two global pandemics, COVID-19 and type 2 diabetes mellitus, has been implicated with worse prognosis. The association of diabetes and worse outcome in viral infections stems from the detrimental effect of hyperglycemia to the control of viremia and different components of the host response. This study aimed to describe the epidemiological and clinical characteristics of confirmed COVID-19 patients and establish the association of baseline glycemic status and COVID-19 outcomes among persons with type 2 diabetes.

Methodology. A single center, retrospective study among adult persons with type 2 diabetes diagnosed with COVID-19 in Makati Medical Center from March 1 to August 31, 2020. A total of 156 medical records (26%) out of 584 confirmed cases were reviewed. Data were collected on diabetes status, comorbid conditions and laboratory findings. Both Cox proportional hazards models and logistic regression models were fitted. To assess the factors associated with mortality as a dichotomous endpoint (died/survived), binary logistic regression was performed. On the other hand, a time-to-mortality analysis was performed using Cox regression. For the effect estimate, we refer to hazard ratios in the Cox proportional hazards model and odds ratios in the logistic regression models. All analyses were adjusted for age and sex and two models were additionally adjusted for any presence of comorbidity.

Results. A total of 156 COVID-19 patients with diabetes were analyzed. Upon admission, 13% were in diabetic ketosis, 4% were in a state of DKA, and 2% had hypoglycemia. About 5%, 33%, 26%, and 36% of patients had mild, moderate, severe, and critical COVID-19, respectively. Between non-survivors and survivors, the latter group were significantly younger in age (p<.003) and had less ICU admissions (p<.001). Although DKA status upon admission seemed to result in increased odds of non-survival (cOR 5.8 [95% CI 1.1-30.7]), no other feature in the glycemic history was significantly associated with mortality outcome after having adjusted for age and sex. Death in this study was limited to patients with severe or critical disease.

Conclusion. The risk of mortality is five times greater among patients admitted with diabetic ketoacidosis. The incidence of complications were also significantly greater and mortality was limited to patients with severe or critical disease.

Key words: Diabetes mellitus, Coronavirus

INTRODUCTION

Severe respiratory infections have posed serious hazards to global health. In the last two decades, there have been documented major outbreaks of two beta coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002-2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012. These have caused fatal pneumonia with mortality rates as high as 10% and 36%, respectively. In December 2019, a novel coronavirus, subsequently named Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) was discovered in Wuhan, Hubei Province, China that caused clusters of pneumonia cases in the locality. The disease it causes is called COVID-19. Due to rapid sustained human-to-human transmission, the World Health Organization (WHO) declared the COVID-19 outbreak a Public Health Emergency of International Concern on January 30, 2020. This formidable outbreak in many cities in China, expanding internationally, led to the escalation to a pandemic on March 11, 2020.[1]

The pathophysiological mechanisms underlying this condition are still not fully understood, but it has been observed that most severe and fatal cases with COVID-19 have occurred in the elderly or in patients with underlying comorbidities, particularly cardiovascular diseases, diabetes mellitus, chronic lung and renal disease, hypertension and cancer.[2-5] According to one Chinese meta-analysis with 1527 patients, the most prevalent cardiovascular metabolic abnormalities associated with COVID-19 include hypertension (17.1%, 95% CI 9.9-24.4%) and cardio-cerebrovascular disease (16.4%, 95% CI 6.6-26.1%), followed by diabetes (9.7%, 95% CI 6.9-12.5%). It showed that those with diabetes or hypertension had a 2-fold increase in risk of severe disease or requiring intensive care unit (ICU) admission.[6]

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Corresponding author: Marion B. Sarigumba, MD
Section of Endocrinology, Diabetes and Metabolism,
Department of Medicine Makati Medical Center
No. 2 Amorsolo St., Legaspi Village, Makati City 1229
Tel. No.:+632-84207460
E-mail: marionsarigumba@gmail.com
ORCiD: https://orcid.org/0000-0002-7913-6941

The coexistence of these two global pandemics, COVID-19 and type 2 diabetes mellitus, has been implicated with worse prognosis. The association of diabetes and worse outcome in viral infections stems from the detrimental effect of hyperglycemia to the control of viremia and different components of the host response, including the function of immune cells and regulation of cytokines. The aim of this analysis is to describe the epidemiological and clinical characteristics of patients confirmed to have COVID-19 and to establish the association between baseline glycemic status and outcomes of persons with diabetes with COVID-19 infections.

METHODOLOGY

This single-center observational study was approved by the Institutional Review Board of Makati Medical Center (protocol number: MMCIRB 2020-082; date of approval: July 28, 2020).

A list of patients was generated from the Infection Prevention Control Unit (IPCU) of Makati Medical Center by identifying persons with diabetes who were laboratory-confirmed (RT-PCR) to have COVID-19. Medical records of the study population from March 1 to August 31, 2020 were reviewed. Retrospective data review was done on an electronic medical record system.

The subjects were classified according to severity using the WHO COVID-19 Disease Severity Classification (27 May 2020). For each subject, the following data will be gathered: duration of diabetes, Hba1c on admission, presence of diabetes complications (ketosis, ketoacidosis, hyperosmolar hyperglycemic state), oral hypoglycemic and insulin use prior to admission and other comorbidities. The following complications were likewise recorded for each patient: ARDS, Septic Shock, ECMO, Gastro-intestinal Bleeding, Myocarditis or Heart Failure as well as their outcome.

Descriptive statistics was used to summarize the general and clinical characteristics of the subjects. Frequency and proportion were used for categorical variables. Shapiro-Wilk test was used to determine the normality distribution while Levene's test was used to determine the homogeneity of variance of continuous variables. Continuous quantitative data that met the normality assumption was summarized using mean and standard deviation (SD), while those that did not was described using median and range.

Continuous variables which are normally distributed were compared using the Independent t-test. Otherwise, the non-parametric Mann-Whitney U test was used. For categorical variables, Chi-square test was used to compare the outcomes. If the expected percentages in the cells are less than 5%, Fisher's Exact test was used instead.

Both Cox proportional hazards models and logistic regression models were fitted. To assess the factors associated with mortality as a dichotomous endpoint (died/survived), binary logistic regression was performed. On the other hand, a time-to-mortality analysis was performed using Cox regression. For the effect estimate, we refer to hazard ratios in the Cox proportional hazards model and odds ratios in the logistic regression models. All analyses were adjusted for age and sex and two models were additionally adjusted for any presence of comorbidity.

All valid data was included in the analysis. Missing variables were neither replaced nor estimated. Null hypothesis was rejected at 0.05 α-level of significance. STATA 15.0 was used for data analysis.

RESULTS

We analyzed a total of 156 patients with diabetes (26%) out of 584 COVID confirmed cases. One subject was excluded due to history of solid organ transplantation. Of these, 25 (16%) expired. Most were male (63%), the mean (±SD) age was 60±13 years, and median BMI was 27 (range 18-52) kg/m2. About 1 in 5 patients were admitted to ICU (17%). The most common comorbidities besides diabetes were hypertension (76%) and chronic kidney disease (17%).

Upon admission, 13% were in diabetic ketosis, 4% were in a state of DKA, and 2% had hypoglycemia. The median baseline diabetes duration, HbA1c, and CBG of patients were 5 (range 0-51) years, 7.52 (range 4.79-18.42) %, and 196 (range 61-568) mg/dl, respectively. About 66% were on oral hypoglycemic agents (OHA), while 14% were using injectables. Comparing the non-survivors with the survivors, they differed in terms of age and need for ICU admission. About 5%, 33%, 26%, and 36% of patients had mild, moderate, severe, and critical COVID-19, respectively.

Between non-survivors and survivors, the latter group were significantly younger in age ($p< .003$) and had less ICU admissions ($p< .001$) (Table 1).

Patient complications in decreasing order were pneumonia (92%), renal failure (47%), and ARDS (26%), and shock (10%). There were significantly higher proportions of renal failure (68% vs 44%), ARDS (84% vs 15%), and shock (32% vs 5%) among those who did not survive. The median durations of hospital for survivors and non-survivors were 13 (range 0-30) and 9 (range 1-26) days ($p=.064$), respectively (Table 2).

Although DKA status upon admission seemed to result in increased odds of non-survival (cOR 5.8 [95% CI 1.1-30.7]), no feature in the glycemic history was significantly associated with mortality outcome after having adjusted for age, sex and any comorbidity (Table 3).

Among patients with severe or critical COVID-19, all except 2 developed pneumonia, more than 7 in 10 were intubated, just over 6 in 10 suffered renal failure, and about 4 in 10 were complicated by ARDS. These incidences were significantly greater compared to their counterparts in the mildly to moderately ill (respectively 83%, 3%, 25%, and 3%). Death in this study was limited to patients with severe or critical disease.

Cox regression

Cox proportional hazard model was estimated to determine the association of glycemic control to time to mortality. Hazard ratios and the corresponding 95% confidence intervals were reported (Figure 1).

Table 1. Characteristics of COVID-19 patients with diabetes mellitus (n=156)

	Overall (n=156)	Non-survivors (n=25)	Survivors (n=131)	*p*
	Mean ± SD; Frequency (%); Median (Range)			
Age (years)	59.83 ± 13.26	66.96 ± 14.99	58.47 ± 12.60	**<.003***
Sex				.501†
Male	98 (62.82)	14 (56)	84 (64.12)	
Female	58 (37.18)	11 (44)	47 (35.88)	
BMI (kg/m^2)	27.02 (18.14–52.22); [n=116]	28.34 (18.79–40.9); [n=17]	27 (18.14–52.22); [n=99]	.325‡
Need for ICU admission				**<.002**§
ICU	26 (16.67)	10 (40)	16 (12.21)	
Non-ICU	130 (83.33)	15 (60)	115 (87.79)	
Comorbidities				
Hypertension	119 (76.28)	21 (84)	98 (74.81)	.322†
CKD	26 (16.67)	6 (24)	20 (15.27)	.377§
CVD	11 (7.05)	3 (12)	8 (6.11)	.385§
Cancer	5 (3.21)	1 (4)	4 (3.05)	.588§
Others	19 (12.18)	4 (16)	15 (11.45)	.511§
Baseline glycemic status				
Duration of diabetes (years)	5 (0–51); [n=134]	5 (0–51); [n=19]	5 (0–30); [n=115]	.614‡
<5	58 (43.28)	6 (31.58)	53 (45.22)	.286§
5 – 10	54 (40.30)	11 (57.89)	43 (37.39)	
>10	22 (16.42)	2 (10.53)	20 (17.39)	
HbA1c (%)	7.52 (4.79–18.42); [n=153]	6.84 (5.35–12.19); [n=24]	7.59 (4.79–18.42); [n=129]	.192‡
<9	98 (64.05)	16 (66.67)	82 (63.57)	.771†
≥9	55 (35.95)	8 (33.33)	47 (36.43)	
Initial CBG (mg/dl)	196 (61–568)	188 (61–401)	196 (71–568)	.643‡
<180	59 (37.82)	10 (40)	49 (37.40)	.806†
≥180	97 (62.18)	15 (60)	82 (62.60)	
Admission status				
DKA	6 (3.85)	3 (12)	3 (2.29)	.053§
Diabetic ketosis	20 (12.82)	3 (12)	17 (12.88)	1.000†
Hypoglycemia	3 (1.92)	1 (4)	2 (1.53)	.410§
Medications				.019‡
None	47 (30.13)	5 (20)	42 (32.06)	
OHA only	87 (55.77)	14 (56)	73 (55.73)	
Injectables only	6 (3.85)	4 (16)	2 (1.53)	
Both	16 (10.26)	2 (8)	14 (10.69)	
COVID-19 severity				<.001§
Mild	7 (4.49)	0	7 (5.34)	
Moderate	52 (33.33)	0	52 (39.69)	
Severe	41 (26.28)	0	41 (31.30)	
Critical	56 (35.90)	25 (100)	31 (23.66)	

Statistical Tests Used: *–Independent t-test; †–Chi-square test; ‡–Mann Whitney U test; §–Fisher's Exact test.

Table 2. Complications and duration of hospital stay among patients (n=156)

	Overall (n=156)	Non-survivors (n=25)	Survivors (n=131)	*p*
	Frequency (%); Median (Range)			
Pneumonia	144 (92.31)	25 (100)	119 (90.84)	.216§
Renal failure	74 (47.44)	17 (68)	57 (43.51)	.025†
ARDS	41 (26.28)	21 (84)	20 (15.27)	<.001†
Shock	15 (9.62)	8 (32)	7 (5.34)	<.001§
Gastrointestinal	2 (1.28)	1 (4)	1 (0.76)	.296§
Heart failure or myocarditis	1 (0.64)	0	1 (0.76)	1.000§
Seizure	0	0	0	-
Hospital days	13 (0–30)	9 (1–26)	13 (0–30)	.064‡

Statistical Tests Used: †–Chi-square test; ‡–Mann Whitney U test; §–Fisher's Exact test.

The risk of mortality is five times higher among patients admitted with diabetic ketoacidosis. No other feature in the glycemic history was significantly associated with hazard of mortality on crude analysis.

The median survival time across all patients was estimated at no later than 26 days from admission, based on the 95% confidence intervals of survival probabilities, which should contain 50% survivorship.

DISCUSSION

This present study demonstrates that the risk of mortality is five times higher among patients admitted with diabetic ketoacidosis. No other features in the glycemic history was significantly associated with mortality outcome after having adjusted for age, sex and any comorbidity. The incidence of complications were significantly greater and mortality was limited to patients with severe or critical disease. The non-survivors and survivors differed in terms of age and need for ICU admission.

Table 3. Association between glycemic history and mortality (n=156)

	Crude OR (95% CI)	*p*	Adjusted[a] OR (95% CI)	*p*	Adjusted OR[c] (95% CI)	*p*
DM history						
Duration of diabetes (years)						
<5	Reference	-	Reference	-	Reference	-
5–10	2.217 (0.76–6.49)	.146	1.401 (0.44–4.43)	.566	1.376 (0.43-4.36)	.588
>10	0.867 (0.16–4.66)	.868	0.425 (0.07–2.64)	.359	.409 (0.07-2.56)	.340
HbA1c, ≥9%	0.872 (0.45–2.19)	.771	1.295 (0.48–3.48)	.609	1.308 (0.48-3.53)	.596
Initial CBG, ≥180 mg/dl	0.896 (0.37–2.15)	.806	1.207 (0.48–3.03)	.689	1.224 (0.48-3.09)	.669
Admission status						
Any complication	1.926 (0.72–5.16)	.192	2.070 (0.73–5.83)	.169	2.015 (0.71-5.71)	.187
DKA	**5.818 (1.10–30.69)**	**.038**	5.186 (1.01–1.09)	.063	4.650 (0.82-26.46)	.083
Diabetic ketosis	0.914 (0.25–3.39)	.894	1.148 (0.29–4.56)	.845	1.168 (0.29-4.68)	.827
Hypoglycemia	2.688 (0.23–30.82)	.427	1.658 (0.13–21.14)	.697	1.562 (0.12-19.82)	.731

[a] Model adjusted for age and sex; [b]Model adjusted for age, sex and any comorbidity

Table 4. Comparison of clinical outcomes by COVID-19 severity (n=156)

	Overall (n=156)	Mild/Moderate (n=59)	Severe/Critical (n=97)	*p*
		Frequency (%)		
Pneumonia	144 (92.31)	49 (83.05)	95 (97.94)	**<.001**
Renal failure	74 (47.44)	15 (25.42)	59 (60.82)	**<.001**
ARDS	41 (26.28)	2 (3.39)	39 (40.21)	**<.001**
Shock	15 (9.62)	0	15 (15.46)	**<.001**
Gastrointestinal	2 (1.28)	0	2 (2.06)	.527
Heart failure or myocarditis	1 (0.64)	0	1 (1.03)	1.000
Death	25 (16.03)	0	25 (25.77)	**<.001**

Statistical Tests Used: Chi-square test/Fisher's Exact test.

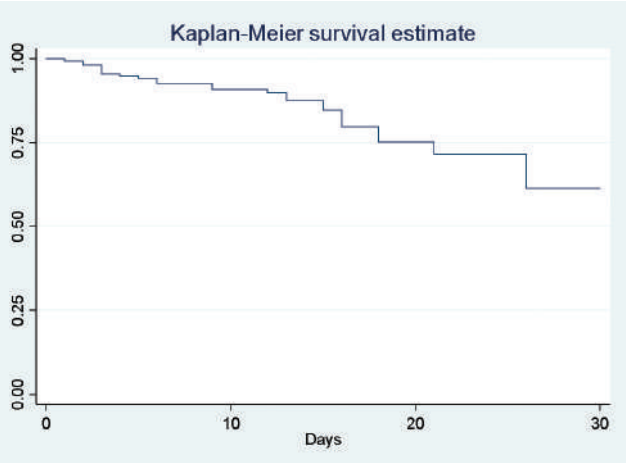

Figure 1. Kaplan-Meier curve of survival probability.

The findings are consistent with the study done by Pal et al., that DKA in COVID-19 patients portend a poor prognosis with a mortality rate approaching 50%.[7] An investigation in the United States by Bode et al., showed that those with uncontrolled hyperglycemia had a longer length of hospital stay than cohorts with good glycemic control.[8] NHS England suggested that patients with both controlled and uncontrolled diabetes with COVID-19, have a significant increase in death in comparison to cohorts without diabetes even after adjusting possible confounders.[9] Zhu et al., analyzed the largest diabetic COVID-19 cohort so far involving 9,663 patients in China, and found unequivocal results to implicate diabetes mellitus in higher risk of death and other detrimental outcomes of COVID-19.[10] Nevertheless, the Chinese Centre for Disease Control and Prevention reported a case fatality rate (CFR) of 7.3% in patients with diabetes, compared to a CFR of 2.3% of overall population of 44,672 patients with COVID-19.[11]

There are limited studies to date which analyzed the outcomes of COVID-19 based on severity, stratified on the baseline glycemic control in patients with diabetes. To the best of our knowledge, this is the first study conducted in the Philippine setting to determine the association of baseline glycemic status and outcome of persons with type 2 diabetes with COVID-19 infections.

SARS-CoV binds to ACE2 in the pancreatic islets leading to islet damage, and acute diabetes. This interaction leads to insulinopenia and increased risk of diabetic ketoacidosis (DKA), especially in patients with pre-existing diabetes. Interleukin-6 is an important cytokine of the hyper-inflammatory state in COVID-19 which has been found to be elevated in DKA and serves as a driver of ketogenesis. Co-existence of DKA in COVID-19 may pose an increased risk over other infectious diseases of equivalent severity.[12] Furthermore, diabetes is associated with the activation of the renin-angiotensin system in different tissues. SARS-CoV-2 utilizes angiotensin-converting enzyme 2 (ACE2) to bind and gain entry to infected cells and reduces the expression of ACE2. Beta cell injury has been implicated in its pathogenesis, leading to viral 'sepsis' which could induce resistance to action of insulin posing additional challenges to management.[13]

An effort to efficiently manage uncontrolled glycemia is strongly advocated with an aim to lower morbidity and mortality. Economic problems exacerbated by the lockdown and COVID-19 has potentially led to non-compliance to pre-admission treatment regimens. Other factors which could affect glycemic control among patients during this pandemic include disorderly lifestyles with consequent weight gain, the lack of readily available access to contact their physicians, and fear of contracting the infection by clinic visits. The increased risk of mortality as found in this study in patients with hyperglycemic

crisis at presentation should encourage us all to achieve aggressive glycemic control.

As with any retrospective review, there are limitations in the data available. The single-center study design with a relatively small sample size is inherently prone to bias. There is also an unprecedented scale of the COVID-19 pandemic, thus, full pre-hospital status of diabetes mellitus from the current cohort were not retrieved due to urgent circumstances. Interestingly, Bode et al., reported a significantly higher percentage of in-patients with COVID-19 who had uncontrolled hyperglycemia but were not diagnosed as diabetes. This suggests that stress hyperglycemia may have a worse outcome in ICU, compared to a known patient with diabetes.[13] It was also noteworthy in some studies that history of microvascular and macrovascular complications was independently associated with risk of death. However, these are factors which were not included in the present study and should be looked into as a future research direction.

CONCLUSION

In conclusion, the risk of mortality is five times higher among patients admitted with diabetic ketoacidosis. The incidence of complications were significantly greater and mortality was limited to patients with severe or critical disease. Although DKA status upon admission seemed to result in increased odds of non-survival, no other feature in the glycemic history was significantly associated with mortality outcome after having adjusted for age and sex in this study.

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

Author Disclosure
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ORIGINAL ARTICLE

Calcium, Vitamin D, and Bone Derangement in Nephrotic Syndrome

Samantha Peiling Yang,[1,2] Lizhen Ong,[3] Tze Ping Loh,[3] Horng Ruey Chua,[4] Cassandra Tham,[2] Khoo Chin Meng,[1,2] Lim Pin[1,2]

[1]*Endocrinology Division, Department of Medicine, National University Hospital, Singapore*
[2]*Endocrinology Division, Yong Loo Lin School of Medicine, Singapore*
[3]*Department of Laboratory Medicine, National University Hospital, Singapore*
[4]*Nephrology Division, Department of Medicine, National University Hospital, Singapore*

Abstract

Introduction. Derangement in calcium homeostasis is common in nephrotic syndrome (NS). It is postulated that low serum total calcium and vitamin D levels are due to loss of protein-bound calcium and vitamin D. It is unclear if free calcium and free vitamin D levels are truly low. The guideline is lacking with regards to calcium and vitamin D supplementation in NS. This study aims to examine calcium and vitamin D homeostasis and bone turnover in NS to guide practice in calcium and vitamin D levels supplementation.

Methodology. This is a prospective pilot study of ten patients diagnosed with NS, and eight healthy controls. Calcium, vitamin D, and bone turnover-related analytes were assessed at baseline, partial and complete remission in NS patients and in healthy controls.

Results. NS patients had low free and total serum calcium, low total 25(OH)D, normal total 1,25(OH)D levels and lack of parathyroid hormone response. With remission of disease, serum calcium and vitamin D metabolites improved. However, nephrotic patients who do not attain complete disease remission continue to have low 25(OH)D level.

Conclusion. In this study, the vitamin D and calcium derangement observed at nephrotic syndrome presentation trended towards normalisation in remission. This suggested calcium and vitamin D replacement may not be indicated in early-phase nephrotic syndrome but may be considered in prolonged nephrotic syndrome.

Key words: Vitamin D deficiency, hypocalcaemia, bone loss, immune-mediated nephrotic syndrome

INTRODUCTION

Derangement in calcium homeostasis is common in nephrotic syndrome. It is postulated that the low serum total calcium and vitamin D levels in nephrotic syndrome are due to the loss of protein-bound calcium and vitamin D respectively. However, it is not clear if free calcium and free vitamin D levels are truly low.[1-5] Low serum free calcium had been shown in some studies in nephrotic syndrome[3,5,6] but not in others.[2,4] Whereas, low vitamin D level had been shown to be due to the loss of 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25-(OH)D], which are mostly protein-bound, in the urine.[7-8]

Also, patients with immune-mediated nephrotic syndrome are frequently treated with immunosuppressants such as steroids, cyclosporine, or tacrolimus that could affect bone resorption and formation. The current guidelines[9-10] with regards to calcium and vitamin D metabolism and supplementation in nephrotic syndrome, with normal renal function, is lacking. There is variable practice in the monitoring of calcium and vitamin D levels and supplementation.

This study aims to examine the calcium and vitamin D homeostasis and the impact on bone turnover in patients with nephrotic syndrome during treatment and remission.

METHODOLOGY

Patients

This was a single-centre, prospective pilot study of ten adult patients diagnosed with nephrotic syndrome, and eight healthy controls. The diagnosis of nephrotic syndrome was based on clinical presentation and renal biopsy findings, including that from light microscopy, immunofluorescence, and electron microscopy. The exclusion criteria included: (a) patients with acute kidney injury, (b) stage 3B and above chronic kidney disease (i.e., estimated GFR <45 ml/min), (c) diabetic nephropathy, (d) use of corticosteroids, vitamin D or calcium supplements in the last 6 months prior to study commencement. Duration of nephrotic syndrome was defined as time of study recruitment to time of complete remission, or time of study cessation (28 February 2017) if not in remission by then. We conducted this study over 3 years and 8 months. The study was approved by National Healthcare Group domain specific review board

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Corresponding author: Samantha Peiling Yang, MBBS, MRCP
Senior Consultant
Division of Endocrinology, Department of Medicine
National University Hospital, 1E Kent Ridge Rd, Singapore 119228
Tel no: (+65) 9127 6805
E-mail: mdcyp@nus.edu.sg
ORCiD: https://orcid.org/0000-0003-0838-6721

ethics committee (reference number: NHG DSRB Ref: 2013/00590). All study subjects provided written consent before enrolment into the study.

Biochemistry

Serum and urine biochemical markers were assessed (i) at baseline prior to starting treatment, (ii) during partial remission, as defined by reduction in proteinuria by 50% or sub-nephrotic range proteinuria, about 2 to 6 weeks into the treatment with immunosuppressant, and (iii) during complete remission as defined by proteinuria <0.3 g/day using 24 hr urinary collection or urine protein to creatinine ratio. The timing of assessment for partial & complete remission could be variable depending on the clinical course of each nephrotic patient.

Fasting venous blood was collected in a heparinised blood gas syringe and sent on ice for ionised calcium measurement within 15 minutes of collection to ensure sample integrity. Serum total calcium, magnesium, phosphate, albumin, creatinine were measured on the Advia 2400 analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). Serum ionized calcium was measured using ion selective electrode on Siemens Rapidlab 1265. Serum total 1,25(OH) D was measured by liquid chromatography-tandem mass spectrometry (LCMS) assay at Mayo Medical Laboratories. Serum total 25(OH)D was measured by: (a) liquid chromatography-tandem mass spectrometry assay at Shimadzu Laboratory, Singapore and (b) competitive protein binding immunoassay on Roche Elecys e411. Plasma fibroblast growth factor-23 (FGF-23) was measured on ELISA assay at Mayo Medical Laboratories. Serum vitamin D binding protein was measured by quantitative sandwich enzyme immunoassay using the Quantikine ELISA kit. The 24-hour urinary total calcium, magnesium, phosphate, creatinine and protein was measured on the Advia 2400 analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). One of the nephrotic subjects declined 24-hour urinary collection and only agreed to proceed with a spot urine protein, creatinine, calcium, magnesium, and phosphate.

Serum C-terminal telopeptides (CTX), plasma intact parathyroid hormones (iPTH), and serum alkaline phosphatase (ALP), were evaluated as markers of bone turnover. Serum iPTH was assessed using 2nd generation chemiluminescent immunoassay on Beckman DXI 800 (Beckman Coulter, Inc., USA) from April 2014; the initial 5 iPTH samples were measured on Advia Centaur analyser. Serum CTX was measured on Roche Cobas E411-based on ECLIA (Electro-ChemiLuminescence Immunoassay) and serum ALP on Beckman DXI 800 (Beckman Coulter, Inc., USA).

Lifestyle survey

Both subjects with nephrotic syndrome and healthy volunteers were interviewed using a standardized questionnaire (Appendix 1). This provided information on their dietary calcium and vitamin D content and sun exposure.

Statistical methods

For sample size calculation, utilizing paired t test to compare assumed mean ionised calcium difference of 0.1mmol/L between nephrotic syndrome at presentation and in remission, assuming standard deviation of the difference of ±0.1 mmol/L (based on our previous study),[6] with an alpha of 0.05, power of 80%, and an allocation ratio of 1:1, a total of 10 people per group should be recruited.[11] We compared the biochemical markers across the 3 time points of nephrotic syndrome status (baseline, partial remission, complete remission) using a repeated measure fixed-effect model with a generalised estimating equation (exchangable correlation structure, Gaussian identity, with robust variance). Parametric variables were reported as mean with standard deviation, and compared using t-test; non-parametric variables were reported as median with range and compared using Mann-Whitney U test. We compared the biochemical markers between baseline nephrotic syndrome state and healthy volunteers, as well as complete remission state and healthy volunteers using T-test. Categorical variables were reported in frequency (percentage) and compared using Fisher's exact chi-square test. A 2-tailed p-value <0.05 was considered statistical significance. All analysis was performed using Stata software (Version 15.1; StataCorp, Texas, USA).

RESULTS

Baseline characteristics of subjects

The 10 nephrotic patients had proteinuria (mean urinary total protein 8.2±3.4 gm/day), with hypoalbuminaemia, and normal glomerular filtration rate (median 73 ml/min, range 55 to 136 ml/min). (Table 1) Majority (80%, 8/10) of the patients received immunosuppressants as monotherapy or combination therapy as clinically indicated. Nine of the nephrotic patients were newly diagnosed on study recruitment, whereas 1 nephrotic patient had nephrotic syndrome relapse for 3 years on study recruitment.

There were eight healthy controls in this study with normal serum creatinine (median 62 umol/L, reference range 60-107 umol/L). The healthy volunteers were younger and were mainly female as compared to nephrotic patients though the median age and gender of nephrotic patients were not statistically different as compared with healthy volunteers. On review of lifestyle factors, both the nephrotic patients and healthy volunteers had low median daily vitamin D and calcium intake.

Biochemical variables with disease progress

Both free calcium and total calcium were significantly lower at nephrotic disease presentation compared to controls. (Table 2). However, there was a lack of iPTH response to hypocalcaemia. Both free and total calcium improved significantly with complete disease remission.

Total 25(OH)D and 1,25(OH)D were significantly lower at baseline compared to healthy controls. These improved significantly with complete disease remission (Figure 1). With complete disease remission, the serum vitamin D binding protein and serum albumin increased significantly from baseline. At baseline, total 1,25(OH)D correlated with total 25(OH)D level measured by mass spectrometry (r=0.6624, p 0.04), and total 25(OH)D level measured by immunoassay (r=0.930, p 0.01).

Out of the 10 nephrotic patients, 4 had persistent nephrotic syndrome for more than 6 months duration, and 5 did not attain complete disease remission by the end of the study.

Table 1. Characteristics of patients and healthy controls

Characteristics	Nephrotic Syndrome patients (n=10)	Healthy controls (n=8)	P value
Age at study entry (years) [median (range)]	56.4 (25.6 – 70.3)	39.8 (25.9 – 57.6)	0.131
Gender			0.637
Males	6 (60.0%)	3 (37.5%)	
Females	4 (40.0%)	5 (62.5%)	
Ethnicity			1.000
Chinese	6 (60.0%)	5 (62.5%)	
Malay	1 (10.0%)	0 (0%)	
Indian	1 (10.0%)	1 (12.5%)	
Others	2 (20.0%)	2 (25%)	
Duration of Nephrotic Syndrome (months) [median (range)]	4.54 (1.78 – 27.35)		
Etiology of Nephrotic Syndrome			
Membranous nephropathy	5 (50.0%)		
Minimal change disease	4 (40.0%)		
Focal segmental glomerulosclerosis	1 (10.0%)		
Immunosuppressant therapy received[a]			
Prednisolone	8 (80.0%)		
Cyclosporin	5 (50.0%)		
Cyclophosphamide	3 (30.0%)		
Tacolimus	1 (10.0%)		
Rituximab	1 (10.0%)		
Lifestyle factors			
Dietary vitamin D intake per day (IU) [median (range)]	233 (21 – 1798)	384 (8 – 3037)	0.753
Dietary calcium intake per day (mg) [median (range)]	523 (244 – 1009)	273 (152 – 840)	0.133
Sun exposure per week (hours) [median (range)]	2.7 (0 – 8.0)	2.0 (0 – 7.3)	0.247

[a]Some patients had received several types of immunosuppressant and were included in multiple categories.

Vitamin D level is lower in nephrotic syndrome patients with nil or partial remission as compared to those who attain complete remission (p 0.04) as illustrated in Figure 2.

The serum intact-parathyroid hormone (iPTH), serum magnesium, serum phosphate, serum fibroblast growth factor-23 (FGF-23), serum c-terminal telopeptide (CTX), and alkaline phosphatase (ALP) levels did not differ significantly at baseline when compared to healthy controls, and did not change significantly with treatment.

Bone turnover markers in relation to calcium, vitamin D, and albumin status

In nephrotic syndrome at disease presentation, lower total 25(OH)D level (measured by mass spectrometry) was associated with higher iPTH (r=-0.636, p<0.05) and CTX levels (r=-0.590, p=0.073, trend towards significance), and lower ALP levels (r=0.555,p=0.096), trend towards significance). This is likely related to increased bone resorption at diagnosis, but the 25-OH vitamin D level improved with steroid therapy and disease remission.

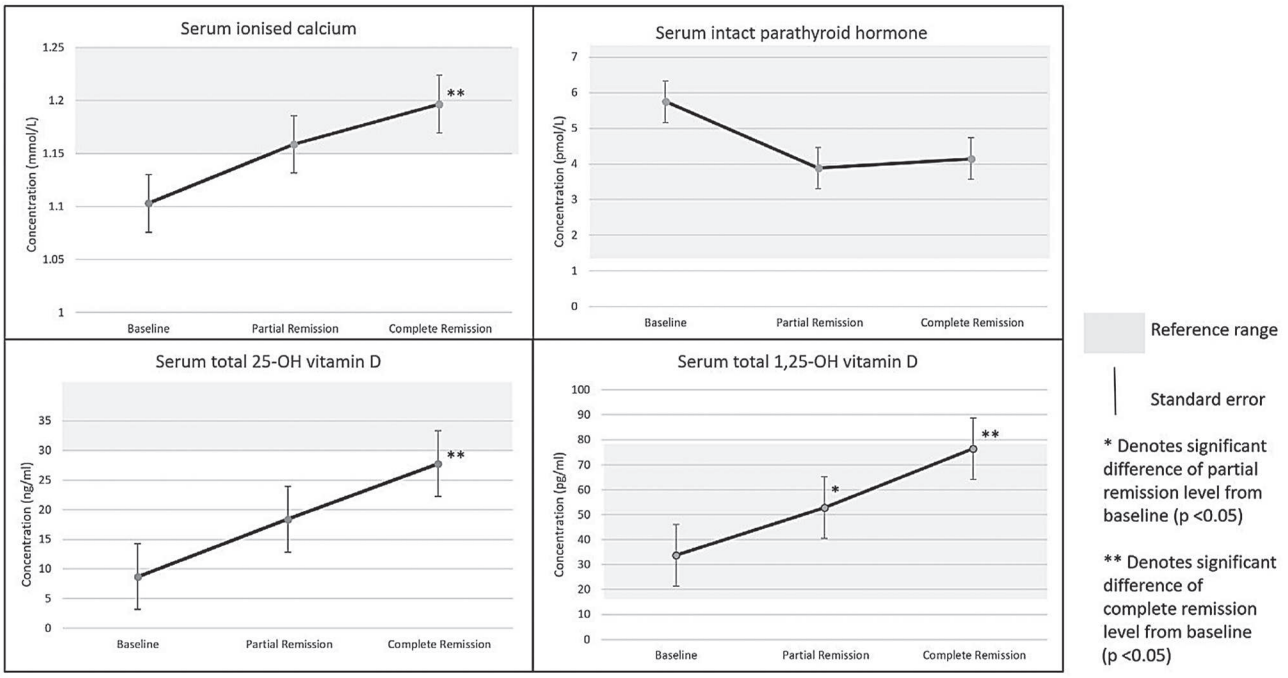

Figure 1. Calcium, parathyroid hormone, 25(OH)D (by mass spectrometry), and 1,25(OH)D levels with nephrotic syndrome remission.

Table 2. Comparison of biochemical variables in 3 phases of nephrotic syndrome (NS) and healthy control

Biochemical variables	Reference range	Baseline NS (mean, SD) [n=10]	Partial remission NS (mean, SD) [n=8, includes data of the 5 NS who attain complete remission, when they were in partial remission phase]	Complete remission NS (mean, SD) [n=5]	Healthy control (mean, SD) [n=8]	Correlation in NS patients across 3 stages of disease state (Baseline, partial, & complete remission NS) (p value)	Baseline NS vs healthy control (p value)	Complete remission NS vs healthy control (p value)
Calcium and related analytes								
Ionised serum calcium (mmol/L)	1.15 - 1.35	1.10 (0.08) ⇩	1.16 (0.09) ⇔	1.20 (0.04) ⇔	1.17 (0.02) ⇔	<0.001	0.034	0.066
Total serum calcium (mmol/L)	2.15 - 2.55	1.98 (0.15) ⇩	2.23 (0.12) ⇔	2.35 (0.13) ⇔	2.26 (0.05) ⇔	<0.001	0.0001	0.113
Serum phosphate (mmol/L)	0.85 - 1.45	1.30 (0.35) ⇔	1.00 (0.25) ⇔	1.19 (0.32) ⇔	1.15 (0.10) ⇔	0.301	0.209	0.759
Intact-parathyroid hormone (pmol/L)	1.3 - 9.3	5.8 (2.9) ⇔	3.9 (1.1) ⇔	3.9 (1.3) ⇔	6.1 (1.5) ⇔	0.065	0.780	0.020
Serum magnesium (mmol/L)	0.75 - 1.07	0.78 (0.13) ⇔	0.84 (0.04) ⇔	0.80 (0.05) ⇔	0.84 (0.06) ⇔	0.532	0.225	0.309
Serum albumin (g/L)	38 - 48	22.6 (5.4) ⇩	35.1 (6.0) ⇩	40.4 (4.8) ⇔	44.6 (2.6) ⇔	<0.001	$2.83x10^{-8}$	0.061
Serum creatinine (umol/L)	60 - 107	83 (22) ⇔	78 (22) ⇔	76 (23) ⇔	62 (11) ⇔	0.502	0.022	0.256
24 hour-urinary calcium (mmol/day)	2.5-10.0	1.1 (0.8) ⇩	3.3 (2.0)	3.8 (1.9)	-	<0.001	-	-
24 hour-urinary magnesium (mmol/day)	6-10	2.4 (1.5) ⇩	3.0 (1.0) ⇩	3.0 (1.5) ⇩	-	<0.001	-	-
24 hour-urinary phosphate (mmol/day)	5-50	14.0 (7.2) ⇔	17.4 (6.0)	14.2 (9.0) ⇔	-	0.986		
Vitamin D-related analytes								
Total serum 25(OH)D by mass spectrometry (ng/ml)	Deficient: < 20 Insufficient: 20 - 29 Sufficient: 30 - 100	8.7 (4.7) ⇩	18.4 (8.8) ⇩	27.8 (7.2) ⇩	18.0 (6.4) ⇩	<0.001	0.004	0.027
Total serum 25(OH) D by immunoassay (ng/ml)	Deficient: < 20 Insufficient: 20 - 29 Sufficient: 30 - 100	6.4 (4.5) ⇩	8.1 (3.9) ⇩	15.7 (5.1) ⇩	15.6 (6.4) ⇩	<0.001	0.008	0.964
Total serum 1,25(OH)D by mass spectrometry (pg/ml)	Male: 18-64 Females: 18-78	33.7 (17.0) ⇔	52.9 (11.0) ⇔	81.2 (51.6) females ⇔ males ⇧	59.9 (13.5) ⇔	<0.001	0.002	0.280
Vitamin D binding protein (ug/ml)	55.9 - 473.0	174.5 (66.2) ⇔	244.6 (71.6) ⇔	256.2 (46.2) ⇔	253.5 (114.4) ⇔	0.005	0.114	0.955
Bone turnover analytes								
C-terminal telopeptide (ug/L)	Pre-menopausal: 0.070 - 0.670 Menopausal: 0.080 - 0.810 Male: 0.154 - 0.885	0.487 (0.204) ⇔	0.572 (0.288) ⇔	0.667 (0.439) ⇔	0.363 (0.224) ⇔	0.439	0.243	0.123
Alkaline phosphatase (U/L)	40 - 130	78 (18) ⇔	69 (21) ⇔	92 (33) ⇔	66 (13) ⇔	0.784	0.127	0.066

The arrows indicate if the variables are normal, low, or high with respect to the reference ranges. Shaded p values are ≤ 0.05.

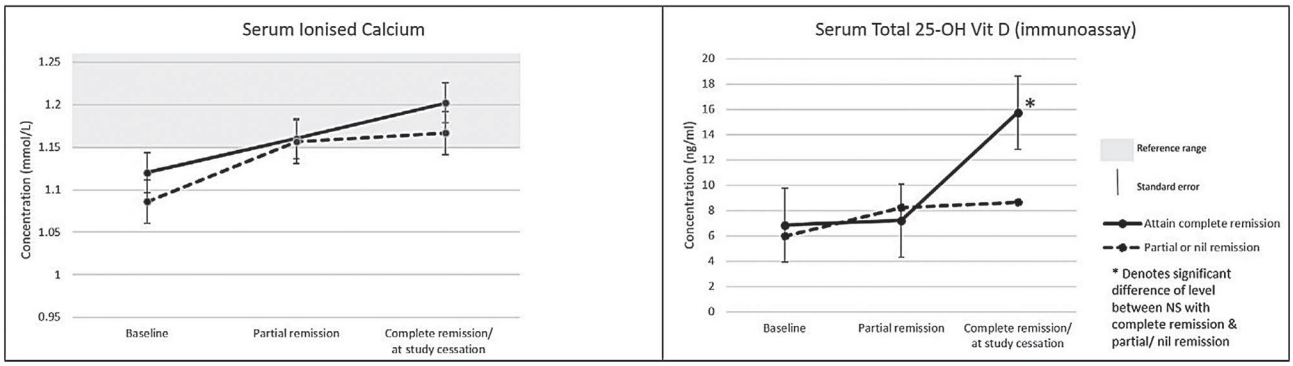

Figure 2. Calcium and 25(OH)D levels between nephrotic syndrome with complete remission and partial or nil remission.

In the eight nephrotic patients who received steroid therapy, there was no significant differences in iPTH, CTX, and ALP levels between treatment period and baseline. Increased 24-hour urinary calcium excretion was observed as the disease progressed from baseline (mean 1.1±0.8 mmol/day) to partial remission (mean 3.3±2.0 mmol/day) and complete remission (mean 4.2±1.9 mmol/day).

DISCUSSION

In this study, we showed that nephrotic patients had low free and total serum calcium, associated with low total 25-OH vitamin D, normal total 1,25(OH)D levels and lack of iPTH response to hypocalcaemia. With remission of disease, the free and total serum calcium, total 25(OH) D and total 1,25(OH)D levels improved significantly. However, nephrotic patients who do not attain complete disease remission continue to have low 25(OH)D level.

As circulating 25(OH)D and 1,25(OH)D are bound to vitamin D binding protein (85 – 90%) and albumin (10 - 15%), with less than 1% of circulating vitamin D in its free form,[12] the low total 25(OH)D was likely attributed to urinary losses of vitamin D binding protein-bound vitamin D and albumin-bound vitamin D. The urinary losses of vitamin D in nephrotic syndrome had been demonstrated in previous studies.[7,8] Hoof et al., had showed low total 1,25(OH)D (measured by radioreceptor assay) and low free 1,25(OH)D (measured by symmetric dialysis) in 16 nephrotic patients, as compared to 12 healthy volunteers.[13] From our data, in nephrotic patients, the baseline total 1,25(OH)D level was positively correlated with the baseline total 25(OH)D level supporting urinary loss of vitamin D metabolites and reduced 25(OH)D substrates. Even though the total 1,25(OH)D was normal, free 1,25(OH)D could have been low leading to reduced intestinal absorption of calcium contributing to low free serum calcium. In our previous study, we had demonstrated marked impairment of intestinal absorption of calcium amongst nephrotic patients, in whom faecal calcium equalled or exceeded dietary calcium.[1]

Previous studies have shown variable levels of serum free calcium, total 25(OH)D, total 1,25(OH)D, and iPTH.[2-5] Low serum free calcium in 6 nephrotic patients was observed by Malluche et al.,[3] and in our study. In the Tessitore et al., study,[5] only 55.6% of 29 nephrotic patients with normal renal function had low free calcium (In a larger study of 30 nephrotic patients with normal renal function, Mittal et al.,[2] only observed low free calcium in 6.7%. These studies used similar ion selective electrode assay for ionized calcium ascertainment. These studies had utilised immunoassays for measurement of total 25(OH) D[2-5] and Tessitore et al.,[5] measured total 1,25-OH vitamin D using radioimmunoassay (RIA). Some of these vitamin D immunoassays are no longer in use currently. The contemporary 25(OH)D immunoassays have analytical limitations, including poor precision at low concentrations of 25(OH)D.[14] The measurement of vitamin D metabolites using LCMS techniques is now considered the gold standard.[15] In our study, we measured total 25(OH)D and total 1,25(OH) D by mass spectrometry. Contrary to Hoof et al.,[13] we had demonstrated normal total 1,25(OH)D levels in nephrotic patients, similar to previous studies.[2,4,5] Consistent with previous studies,[2-5] we had noted low total 25(OH)D levels, by both immunoassay and mass spectrometry methods, in nephrotic patients. The month of vitamin D measurement was not stated as the study was conducted in a country with tropical climate, having minimal fluctuations in temperature and sun exposure over the course of the year.[16]

Most of the previous studies had measured iPTH using first generation iPTH assays[3-5], except for Mittal et al.,[2] and our study, where second generation iPTH assay was used. Only 1 previous study by Malluche et al., showed elevated iPTH levels in nephrotic patients.[3] Similar to most previous studies,[2,4,5] our study observed inappropriately-normal iPTH levels in nephrotic patients, despite the presence of hypocalcaemia and low vitamin D level, reflecting a lack of PTH response. We postulate the following reasons for this inappropriate PTH response to hypocalcaemia. First, the degree of hypocalcaemia might be mild and insufficient to stimulate hyperparathyroidism. Second, inhibitor of PTH might be present in the nephrotic syndrome. Given that our patients had normal serum magnesium and phosphate, the inhibiting factor for PTH is unknown at present. The low ionised calcium could be contributed by low free 1,25(OH)D leading to reduced intestinal calcium absorption, as well as inadequate PTH response.

Previous studies evaluated skeletal impact of nephrotic syndrome through bone biopsy morphology, reporting features of normal finding, osteomalacia, increased bone turnover or mixed findings.[1-5] Using bone turnover markers assessment, Fujita et al.,[17] showed that urinary deoxypyridinoline (bone resorption marker) increased significantly but serum osteocalcin (bone formation marker) fell significantly in 9 nephrotic patients within 3 months treatment with steroids. There are case series of steroid-induced vertebral fractures and low bone mass in nephrotic syndrome patients treated with steroids with or without cyclosporine.[18,19] However, our study did not find any significant difference in CTX and ALP with treatment of nephrotic syndrome, duration of disease, and between nephrotic patients and healthy controls. This was despite our cohort having a median duration of disease of 4.54 months (range 1.78 – 27.35 months), with majority of patients having received immunosuppressant. The increasing urinary calcium excretion of NS patients in our study with disease remission could be a result of steroid-related hypercalciuria.

Given the bone histology evidence,[1-3,5] bone turnover markers changes[17] reported from previous studies, it is likely that the metabolic bone consequences of nephrotic syndrome could heighten the risk of osteoporosis.[20] However, our study did not show any adverse skeletal biochemical parameters in nephrotic syndrome patients.

This study has several strengths. We had studied nephrotic subjects across several time points during their course of disease allowing for paired comparison. These were also compared with that in healthy individuals. The total 25(OH)D and total 1,25(OH)D were evaluated using mass spectrometry.

The small sample size is a limitation of this study. There is a risk of having false negative results (beta error) from inadequate sampling distribution to make inference. The

healthy volunteers were different from nephrotic patients with the former having lower median age and proportion of males. The median age and gender of nephrotic patients were not statistically different as compared with healthy volunteers due to the small sample size. In addition, we did not measure tissue magnesium level that would have excluded magnesium deficiency more definitively. All the healthy volunteers had vitamin D insufficiency or deficiency, and the nephrotic patients' vitamin D levels were compared against these levels. At complete remission phase, most nephrotic patients still had vitamin D insufficiency or deficiency but were assessed to have no statistical difference in levels compared to our cohort of healthy volunteers.

CONCLUSION

Even though initial nephrotic syndrome stage is associated with low serum calcium and 25(OH)D levels, these resolve with nephrotic disease remission with lack of adverse bone turnover parameters. However, nephrotic patients who do not attain remission continue to have low 25(OH) D level. This suggested calcium and vitamin D replacement may not be indicated in early-phase nephrotic syndrome but may be considered in prolonged nephrotic syndrome.

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

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ORIGINAL ARTICLE

Next Generation Sequencing of 502 Lifestyle and Nutrition related Genetic Polymorphisms reveals Independent Loci for Low Serum 25-hydroxyvitamin D Levels among Adult Respondents of the 2013 Philippine National Nutrition Survey*

Mark Pretzel Zumaraga, Mae Anne Concepcion, Charmaine Duante, Marietta Rodriguez

Department of Science and Technology – Food and Nutrition Research Institute, Bicutan, Taguig City, Philippines

Abstract

Objective. The study determined the relationship of serum vitamin D levels and 502 lifestyle and nutrition related genetic polymorphisms among adult respondents of the 2013 Philippine National Nutrition Survey (NNS).

Methodology. A total of 1,160 adult respondents of the 2013 NNS living in the National Capital Region, Philippines were enrolled. Of the 1,160 sequenced samples, 833 passed the stringent quality control based on multiple parameters and were used for further analysis. Total serum 25-hydroxyvitamin D [25(OH)D] was determined using electro-chemiluminescence binding assay method. Genomic DNA was used for targeted next generation sequencing of 502 lifestyle and nutrition related polymorphisms. Analysis of variance, followed by Tukey post hoc analysis, was employed to compare 25(OH)D serum levels across genotypes.

Results. Of the study participants, 56% was classified as having low serum 25(OH)D. The lower serum 25(OH)D was observed in the following gene/genotypes: *KNG1* rs11924390 T/T; *ANKH* rs2454873 G/G; *NPFFR2* rs4129733 T/G; *SH2B1* rs4788102 G/A; *RAP1A* rs494453 T/T and *CRHBP* rs7728378 T/C. These genes were previously associated to the risk of osteoporosis, obesity, type 2 diabetes mellitus, and stress response.

Conclusion. Large-scale analysis of genes has shown great utility in the discovery of genetic factors that play a role in vitamin D nutrition. Interestingly, loci found in this Filipino population cohort were mostly independent from the canonical vitamin D synthesis and metabolism pathways. Understanding how genetic variations interact with nutrition and lifestyle may aid in the prevention of diseases through screening and identification of susceptible patients who would not benefit from regular supplementation with vitamin D because of genetic alterations and may also be used as basis for future development of functional food enriched with vitamin D.

Key words: Vitamin D, nutrition, nutrigenetics

INTRODUCTION

Vitamin D deficiency (VDD) is a widespread disorder across all age groups around the world. In fact, in addition to infectious diseases and malnutrition, it is among the most prevalent health disorders in recent years.[1] The high prevalence of VDD in a number of countries exists despite the fact that a large number of these countries lie in zones that have sufficient sunlight for vitamin D synthesis including Malaysia, Indonesia and the Philippines.[2] In the Philippines, of the five areas covered during the 2013 National Nutrition Survey (NNS), the highest proportion of deficient and insufficient levels in the country was found in Benguet at 60.3% and lowest in Cagayan at 19.5%. Generally, there were more deficient and insufficient levels among the females compared to the males. Particularly, the lowest mean vitamin D levels were found among women of reproductive age group, 20-39 years old (63.5±1.7 nmol/L).[3]

Historically, VDD has been linked to skeletal diseases, including calcium, phosphorus, and bone metabolism, osteoporosis, fractures, muscle strength, and falls. In the 2000s, growing scientific attention turned to non-skeletal chronic diseases as VDD has been linked to cancer, cardiovascular diseases, metabolic disorders, autoimmune diseases, as well as infectious diseases such as respiratory tract infections.[4] Recently, vitamin D has been

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Corresponding author: Mark Pretzel P. Zumaraga, RCh, MSc
Science Research Specialist II, Department of Science and Technology-Food and Nutrition Research Institute, FNRI Building, DOST Compound, General Santos Avenue, Bicutan, Taguig City, Philippines 1631
Tel. No.: +632-8372071 local 2299
Fax No.: +632-8372934
E-mail: mpzumaraga@yahoo.com
ORCiD: https://orcid.org/0000-0001-5598-8456

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discovered to induce the production of cathelicidins and defensins, which are natural anti-microbials, and has been proposed to prevent and treat coronavirus disease 2019 (COVID-19).[5]

External exposures (sunlight, diet, and vitamin D supplements) are chief determinants of circulating 25(OH) D, the established marker of vitamin D status. However, with several genes controlling pathways that synthesize, transport, and degrade forms of vitamin D, individual genetic variations may also play a role in individual (and potentially population) differences in vitamin D status. Due to inter-individual genetic variation, people who are exposed to a high amount of sunlight or taking high dose of vitamin D supplementation may also suffer from low serum 25(OH)D level. With vitamin D implicated in a wide range of health issues from rickets to cancer and respiratory infections, a fuller understanding of the determinants of vitamin D status is needed and must include consideration of inherited characteristics.[6]

Recent genome-wide association studies (GWASs) of serum 25(OH)D that covered more than 79,366 individuals of European ancestry have identified at least six common genetic variants (minor allele frequency >0.05). These polymorphisms are found in the following genes: (1) the GC (the vitamin D-binding protein gene), (2) the DHCR7/ NADSYN1 region (DHCR7 is involved in a conversion of a 25(OH)D precursor molecule to cholesterol), (3) CYP2R1 and (4) CYP24A1 genes (which encode enzymes involved in 25(OH)D metabolism), as well as (5) SEC23A (Sec23 homolog A, coat protein complex II component involved in endoplasmic reticulum (ER)-Golgi protein trafficking, and (6) AMDHD1 (amidohydrolase domain containing 1) an enzyme involved in the histidine, lysine, phenylalanine, tyrosine, proline, and tryptophan catabolic pathway.[7] Revez et al., further identified 143 independent loci for 25(OH)D in genes involved in lipid metabolism, dermal tissue properties and conjugation of 25(OH)D.[8] Manousaki et al., sought to investigate the phenotypic variance as explained by the genetic variants. One implication of their findings is that the potential genetic influence for vitamin D is instrumenting more than the vitamin D pathway, hence, future studies must focus on interrogating genetic regions (1) not directly involved in 25(OH)D biology; and (2) related to environmental confounders that influence 25(OH)D levels. Additionally, most GWAs have predominantly been focused on European populations, which provide limited information on the usefulness of discovered variants in populations of non- European ancestry.

Although the investigations of whole exomes or even genomes is increasing, sequencing of a specific set of genes (targeted resequencing) is important for research and clinical settings due to its lower cost, maximum coverage of target regions, and more accessible data interpretation.[9] Our study determined the putative relationship of serum 25(OH)D levels with several variations in lifestyle related genes among adult respondents, age 20 years old and above, of the 2013 NNS by targeted resequencing.

This study can be used as basis for a future genetic panel specific to the Filipino population that could assist clinicians in screening and identifying patients who would not benefit from the usual supplementation with vitamin D because of genetic alterations. Our results might also aid in promoting awareness in the scientific community of researchers to identify the involved mechanisms.[9]

METHODOLOGY

Study Subject Ascertainment and Ethical Consideration

This is a sub-study of the vitamin D survey component of the 2013 Philippine NNS. The statistical design used in the vitamin D survey component was a multi-staged stratified sampling design. The first stage of the sampling was the selection of the Primary Sampling Unit (PSU) which consisted of one barangay or a contiguous barangay with at least 500 households. The second stage was the selection of the Enumeration Area (EA) which consisted of a contiguous area in a barangay with 150-200 households and the last stage was the selection of households in the sampled EA that served as the ultimate sampling unit. The survey lasted from June 19 to December 4, 2013 and continued on February 16 to April 15, 2014. For this sub-study, a total of 1,160 adult Filipino respondents from the National Capital Region (NCR) of the 2013 NNS, aged 20–years and above were included. The protocol (code FIERC-2017-002) was approval by the DOST-FNRI Ethics Review Committee for implementation on 31 March 2017.

All the respondents provided written informed consent approving the general research use of their respective specimens for biochemical and DNA analyses, as well as lifestyle information including age, medical history of falls during the past 12 months, use of calcium and vitamin D supplements, calcium intake, serum 25(OH)D, reproductive characteristics, lifestyle behavior and previous history of fragility fracture. Respondents were excluded if they met one of the following (1) female, pregnant and lactating at the time data were collected; and/or (2) did not have complete data on the study variables of interests.

Study Variables

From the household face-to-face interview, the following information on age, sex, race/ethnicity (self-identified), smoking habits, sleeping hours, previous history of fracture (hip and other parts) were collected. Individual food consumption was obtained using a two-day non-consecutive 24- hour Food Recall, one during a weekday and one during a weekend. Individual Dietary Evaluation System (IDES) was used to determine the nutrient content of food items accurately recalled and consumed.

From the examination data, body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. The waist circumference was measured as the perimeter or distance around the natural waist (midway between the lowest rib and the tip of the hip bone) or a measure of the distance around the narrowest portion of the trunk. The tape measure was placed at the midpoint and the respondent was asked to breathe normally; measurement was taken at the end of normal expiration. The hip circumference was measured as the distance around the largest area of the hips, usually the largest part of the buttocks or the greater trochanter. Smoking status was classified as never, current, or former smoker. Blood pressure level was measured using a non-mercurial sphygmomanometer and stethoscope, following standard procedures.[10] Measurements were taken twice.

Total serum 25(OH)D was contracted to an ISO/IEC 15189 accredited laboratory and was determined using the electro-chemiluminescence binding assay method.[11] Briefly, 15 uL of serum sample were incubated with a pretreatment reagent for nine minutes to aid release of bound vitamin D 25(OH)D from its Vitamin D binding protein. The sample was further incubated with a recombinant ruthenium-labeled VDBP to form a complex of Vitamin D (25OH) and ruthenylated-VDBP. Then, with the addition of a biotinylated vitamin D a complex consisting of the ruthenium-labeled VDBP and the biotinylated vitamin D was formed. The entire complex now became bound to the solid phase via interaction of biotin and streptavidin-coated microparticles, which are captured on the surface of the electrode. Voltage was applied to the electrode causing chemiluminescent emission that can be quantified by a photomultiplier. Results were determined via an instrument-specific calibration curve, which is generated by 2-point calibration and a calibration master curve provided via the reagent barcode. The method is standardized against LC-MS/MS, which in turn is traceable to National Institute of Standards and Technology (NIST). The measuring range of the method used is 3.00-70.0 ng/mL (7.50-175 nmol/L), with sensitivity of 4.01 ng/mL (10.0 nmol/L) (CV 18.5 %) and repeatability and intermediate reproducibility values of ≤6.5 % and ≤11.5 %, respectively (https://www.accessdata.fda.gov/cdrh_docs/reviews/K162840.pdf, accessed on November 3, 2020). All blood collections were done inside rooms to avoid exposure of the collected specimen to direct sunlight. In the field, serum was kept frozen in freezers or in ice chests with dry ice. Blood samples in the frozen state were transported to the Biochemical Laboratory (BL) of DOST-FNRI.. These were kept in -80˚C freezers until laboratory analysis was conducted.

Development of SNP panel

A total of 502 variations in lifestyle and diet related genes were selected from literature and the Human Gene Mutation Database (HGMD; http://www.biobase-international.com/product/hgmd). Ion Ampliseq Designer was used to design the Ampliseq Custom Amplicon panel (both Thermo Fisher Scientific, USA) using 275 bp amplicons with UCSC hg19 (https://genome.ucsc.edu/) as the reference genome. Target regions for resequencing comprised the whole exon, exon-intron boundary, and regulatory region (10 kb upstream from the transcription start site) of genes of interest, accounting for a total of 108,670 bp.

Targeted Resequencing of the Nutrigenomics Related Genes

Targeted next generation-sequencing (NGS) remains a popular approach that allows to sequence specific areas of the genome (i.e., selected lifestyle-related genes), for a more rapid and cost-effective mutation analysis than the whole genome sequencing. Briefly, DNA was sequenced using the Ion Proton Next Generation Sequencing or NGS (Invitrogen Life Technologies, USA) according to manufacturer's instructions. For the targeted amplification of 502-genes associated with nutrition-related diseases, the custom Ion Ampliseq™ (Invitrogen Life Technologies, USA), was used. Diluted barcode adapter mix including Ion Xpress™ Barcode Adapter and Ion P1 adaptor were ligated to the end of the digested amplicons with ligase provided in the kit. Adaptor ligated amplicon libraries were purified and subsequently quantified by Quant-iT™ dsDNA HS Assay (Invitrogen, Life Technologies, USA) and Qubit® Fluorometer (Invitrogen, Life Technologies, USA) according to the manufacturers' procedures. After quantification, each amplicon library was equalized and pooled. A template was prepared using the Ion PGM 200 Xpress Template Kit (Invitrogen Life Technologies, USA) and sequencing was performed using the Ion Sequencing kit version 2.0 on an Ion 318 Chip. Preliminary data analysis was done using the Ampliseq™ Variant Caller plug-in within the Ion Torrent Suite software (Invitrogen Life Technologies, USA). Sequencing and downstream bioinformatics analysis were performed at the Philippine Genome Center, University of the Philippines, Diliman, Quezon City.

Bioinformatics and Mutation Calling

Sequencing reads were aligned and filtered using Torrent Suite v3.6.2 (Invitrogen Life Technologies, USA) and the Ampliseq Custom Panel manifest. Variants were annotated and filtered using Torrent Suite v3.6.2. Variant filters were established using a sample set with known mutations and filter settings verified using blinded samples. Annotations were done on variants with quality score >100, read depth ≥30, mutant allele frequency of ≥15%. Filtered variants were visualized in genomic context using IGV (Interactive Genomics Viewer: http:// www.broadinstitute.org/igv/) to exclude sequencing artifacts. There were a total of seven independent sequencing runs conducted to analyze all the samples. Sequencing information of each run is shown in Table 1.

Statistical Analysis

Statistical analysis was performed using the Stata software (version 13.0; Statacorp LLC). Data normality was analyzed using the Kolmogorov-Smirnov test. Normally distributed data were compared using the unpaired *t* test and expressed as means ± SDs. Non–normally distributed data are expressed as medians and IQRs. Between-group comparisons were assessed using the Mann-Whitney *U* test. Pearson's chi-square test was performed to analyze the categorical data. One-way ANOVA, followed

Table 1. Sequencing information of the target regions of the tested samples

Run No.	No. of samples analyzed per run (n)	Total effective read base number (Kb)	Percent read base number on target (%)	Ave. sequencing depth on target	Percent uniformity (%)	No. of variants determined (Ave.)
1	57	119.9	88.1	173.4	92.2	297
2	49	59.1	91.1	67.9	81.5	255
3	77	379.9	96.4	593.3	96.6	325
4	88	334.0	95.8	513.1	96.2	326
5	88	532.0	92.5	790.3	96.0	327
6	203	150.2	92.0	201.9	95.0	319
7	271	242.9	95.9	381.1	95.6	322

by Tukey post hoc test for multiple comparisons, was used to find association between serum 25(OH)D concentration and genotypes. *P-value* of less than 0.05 was *considered significant*. Participants were stratified into two groups according to their vitamin D status: 1) serum 25(OH)D <75 nmol/L, defined as low serum vitamin D, and 2) serum 25(OH)D ≥75 nmol/L, indicating a normal serum vitamin D status.[12]

RESULTS

A total of 1,160 adult respondents of the 2013 NNS adult participated in the study. Of them, 833 passed our inclusion criteria *(See Materials and Methods)*. Four hundred sixty-six (56%) subjects were classified as low (deficient and insufficient) serum 25(OH)D concentration (<75 nmol/mL) and 367 subjects had normal serum 25(OH) D (≥75 nmol/L). Table 2 shows the socio-demographic and anthropometric profiles of study participants, delineating study subjects with low versus normal serum vitamin D concentration. Notably, we found no significant differences in terms of age in years, BMI, waist and hip circumferences and waist-hip ratio between the two groups. Most of the participants with low vitamin D were female (p<0.01).

Diverse environmental factors regulate 25(OH)D metabolism, among which are diet, sun exposure, pollution, and infection. The majority of these factors exert their effects on 25(OH)D concentration by regulating the expression of vitamin D receptor (VDR) protein.[13] Table 3 presents the dietary profile of study participants living in the National Capital Region, Philippines.

Many clinical observations also raised the possibility of a link between vitamin D and risk factors of known metabolic diseases.[14] Table 4 shows the clinical information of study subjects with low and normal vitamin D serum. There were no statistically different means found in systolic, diastolic and sleeping hours, indicating both groups are demographically equivalent. Most participants in both groups were found to be non-smokers, no history of fracture at the hip and other body parts.

Being reliant on an *a priori* hypotheses for gene selection, this study was limited to known gene candidates that may potentially show variation in serum 25(OH)D concentration. Table 5 shows list of genetic variations that were found to have statistically significant difference in serum 25(OH)D concentration across genotypes at p-value <0.05. Our study team call these nutrigenetic (coined from nutrition and genetics) markers of vitamin D nutrition because they are highly sensitive to serum 25(OH)D levels. Indication of lower serum 25(OH)D was observed in the following gene/genotypes (<75 nmol/L, see Table 4): KNG1 rs11924390 T/T; *ANKH* rs2454873 G/G; *NPFFR2* rs4129733 T/G; *SH2B1* rs4788102 G/A; *RAP1A* rs494453 T/T and *CRHBP* rs7728378 T/C. Supplementing with vitamin D or increasing sun exposure could be helpful for people with these genetic variations.[15]

DISCUSSION

Due to the fact that the vitamin D receptor (VDR), the single known regulatory mediator of hormonal vitamin D in higher vertebrates, is expressed in almost every tissue and cell throughout the human body, there have been extensive investigations on potential extra-skeletal effects of vitamin D.[24] This study determined the relationship of serum 25(OH)D concentration with selected lifestyle disease related genes among 1,160 individual NCR respondents of the 2013 Philippine NNS. Of the 1,160 sequenced samples, 833 passed the stringent QC based on multiple parameters and were used for further analysis. S1 Table describes details of the candidate gene regions selected for targeted resequencing. A summary of high-quality variants analyzed for their distribution and association with serum 25(OH)D is provided in S2 Table.

Table 2. Socio-demographic and anthropometric profile of study participants

	Low vit. D (<75 nmol/L) (n=466)	Normal vit. D (≥75 nmol/L) (n=367)	*p-value
Age in years (SD)	41 (16)	47 (15)	0.1455
Sex (n, %)			
Male	148 (32)	220 (60)	<0.0001*
Female	318 (68)	147 (40)	
Ave Body mass index in kg/m^2 SD)	24.0 (4.4)	23.7 (4.2)	0.3203
Ave waist circumference in cm (SD)	81.5 (12.1)	81.8 (11.8)	0.1564
Ave hip circumference in cm (SD)	91.6 (8.8)	90.1 (8.9)	0.2737
Ave waist-to-hip ratio (SD)	0.89 (0.08)	0.91 (0.08)	0.2633

* Pearson's Chi-squared test p-value ≤ 0.05 is considered significant

Table 3. Median nutrient intake of low serum vitamin D vs normal serum vitamin D between study subjects

Nutrient intake	Low vit. D (<75 nmol/L) (n=466)	Normal vit. D (≥75 nmol/L) (n=367)	*p-value
Energy (median, IQR)	1,639.8 (882.2)	1,617.7 (810.7)	0.309
Protein (median, IQR)	53.1 (28.2)	54.5 (29.2)	0.921
Cholesterol (median, IQR)	268.2 (147.9)	271.6 (147.5)	0.546
Fat (median, IQR)	28.3 (32.2)	29.6(26.4)	0.477
Vitamin A (median, IQR)	203.6 (300.1)	215.5 (306.9)	0.921
Thiamin (median, IQR)	0.7 (0.6)	0.7 (0.5)	0.103
Riboflavin (median, IQR)	0.6 (0.4)	0.6 (0.4)	0.309
Niacin (median, IQR)	17.5 (9.1)	18.3 (8.8)	0.685
Vitamin C (median, IQR)	14.7 (28.2)	15.3 (31.5)	0.836
Iron (median, IQR)	7.8 (5.1)	8.7 (5.8)	0.186
Calcium (median, IQR)	253.1 (199.5)	269.7 (224.5)	0.309

* p-value ≤ 0.05 is considered significant

Table 4. Clinical and risk indicators of overall health between low and normal serum vitamin D study participants

	Low vit. D (<75 nmol/L) (n=466)	Normal vit. D (≥75 nmol/L) (n=367)	p-value
Average systolic, mmHg (SD)	118.5 (20)	121.3 (20)	0.1546
Average diastolic, mmHg (SD)	76.7 (12)	78.2 (11)	0.2201
Smoking (n,%)			0.01694
Non-smokers	308 (37)	209 (25)	
Current smokers	92 (11)	100 (12)	
Former smokers	66 (8)	58 (7)	
Sleeping hours (h)	7.3 (1.4)	7.7 (8.5)	0.177
Previous hip fracture (n,%)	2 (0.4)	4 (1)	0.262902
Previous fracture, other body parts (n,%)	18 (3.9)	18 (4.9)	0.462817

Table 5. Nutrigenetic markers found to have significantly different 25(OH)D3 concentration across genotypes

Gene (dbSNP)	Function	Disease pathology/ phenotype	Genotype	No. of study subjects (N=833)	Ave. serum vit. D concentration (SD)	*p-value
[a]KNG1 (rs11924390)	upstream	adiponectin levels[16]	T/T	451	72.4 (27)[a,b]	0.0042
			T/C	256	78.4 (29)[b]	
			C/C	126	79.6 (32)[a]	
[b]ANKH (rs2454873)	intron	Bone mass and geometry[17]	G/G	451	73.0 (27)[a]	0.0377
			G/A	315	78.0 (31)[a]	
			A/A	67	78.4 (26)	
[c]NPFFR2 (rs4129733)	intron	Obesity[18]	T/T	557	76.6 (28)[a]	0.0325
			T/G	236	71.7 (29)[a]	
			G/G	40	78.3 (25)	
[d]SH2B1 (rs4788102)	intron	Obesity[19,20,21]	G/G	586	77.0 (28)[a]	0.0094
			G/A	220	70.4 (26)[a]	
			A/A	27	79.6 (39)	
[e]RAP1A (rs494453)	intron	Osteoporosis[22]	T/T	195	71.4 (27)[a]	0.0277
			T/C	442	75.4 (28)[a]	
			C/C	196	79.1 (31)	
[f]CRHBP (rs7728378)	intron	Stress response[23]	T/T	174	79.0 (32)[a]	0.0433
			T/C	396	72.9 (27)[a]	
			C/C	263	76.6 (27)	

[a]KNG1 - Kininogen-1
[b]ANKH - Ankylosis
[c]NPFFR2 – Neuropeptide FF Receptor 2
[d]SH2B1 – SH2 Domain-Containing Putative Adapter
[e]RAP1A – Ras-related protein
[f]CRHBP – Corticotropin Releasing Hormone Binding Protein
*p-value ≤ 0.05 is considered significant, calculated using ANOVA

Of the study participants, 56% was classified as low serum 25(OH)D. An individual's vitamin D stores are best reflected by serum 25(OH)D which is influenced by diet, age, BMI, sex, skin color, and numerous factors regulating exposure to ultraviolet B radiation. Comparing cases and controls, we found no differences in most of parameters tested in the study, including median nutrient consumption. The NCR is the financial, commercial and industrial center of the Philippines. However, in spite of its urbanized state, the region experiences the effects of the double burden of malnutrition in the form of undernutrition and over nutrition, affecting vulnerable population groups such as children and women (accessed at: https://www.nnc.gov.ph/2-uncategorised/244-ncr-profile; on July 20, 2020). Among the Philippine regions, NCR had the lowest intake of cereals and cereal products (298 grams) particularly rice and rice products (260 grams) and corn and corn products (3 grams), but registered the highest intake in other cereal products (35 grams), which include breads and noodles. A high intake of fish, meat and poultry (246 grams), particularly meat and meat products (96 grams) and poultry (53 grams), was also noted. Other food groups highly consumed in the region were whole milk (46 grams) and vitamin C-rich fruits (13 grams) and beverages (36 grams). However, vegetables, particularly green, leafy, and yellow vegetables (29 grams), and condiments and spices (7 grams) were minimally consumed.[25] Despite evidence for association between 25(OH)D concentration against carbohydrate, protein, and calcium dietary intake, the observed difference did not vary between 25(OH)D classification.

However, it is noteworthy that vitamin D status in females was significantly worse than in males in this group of study subjects (p<0.0001). Natural differences in terms of the amount of subcutaneous fat between males and females could be one of main reasons for the gender difference in serum vitamin D levels, where women have more subcutaneous fat than men. As vitamin D is fat-soluble and the subcutaneous adipose tissue can store large amounts of it, the greater average amount of subcutaneous fat in women takes up more vitamin D molecules leading to fewer vitamin D molecules entering the blood circulation in women than in men.[26]

Traditional twin studies have shown that between the range 50%–80% serum 25(OH)D variability is explained by genetic factors, indicating that it is a highly heritable trait. In fact, our data discovered at least six genetic variations putatively associated with abnormal serum 25(OH)D concentrations (p<0.05). These genes were previously associated to the risk of osteoporosis, type 2 diabetes mellitus, obesity, and stress response.[16-23] Individualized approach, such as vitamin D dietary intervention and/or sun exposure, based on genetic make-up may be helpful for individuals with these genetic variation.[15] Moreover, a particular gene hotspot for serum 25(OH)D concentration, recently discovered through a large-scale GWAS meta-analysis in 2020, was replicated in the present study, the NPFFR2.[27] The Neuropeptide FF (NPFF; with the sequence FLFQPQRF) belongs to the RF-amide peptide family and was first isolated from bovine brain by virtue of the shared N-terminal sequence, RF-NH2.[18] NPFF is ubiquitously expressed in the central nervous system with highest expression in the hypothalamus, posterior pituitary and spinal cord. There are two known NPFF cognate receptors, NPFFR1 (GRP147) and NPFFR2 (GPR74). NPFFR2, a G-protein receptor that binds neuropeptides, is highly expressed in pain-processing regions such as spinal dorsal horn, thalamus and dorsal raphe nucleus.[18] Dahlman et al., have typed four SNPs at this locus and showed that one particular haplotype was protective against obesity.[28] The protective haplotype was associated with higher adipocyte lipid mobilization. NPFFR2 is also known to be closely linked to the GC/VDBP during the whole evolution of vertebrates. VDBP, or Vitamin D binding protein, plays

an important role in the transport and metabolism of vitamin D. In 2019, the first case of a large deletion in the coding portion of the VDBP gene (and adjacent NPFFR2 gene) in a single family was reported. The proband had normal calcium, phosphate and parathyroid hormone levels. When provided with vitamin D supplementation due to very low levels of 25(OH)D, the patient was not responsive even to massive doses of vitamin D (oral or parenteral). The homozygous partial NPFFR2 deletion is of uncertain significance at that time, since the gene has yet to be associated with human disease. Nevertheless, as confirmed from previous GWAS for vitamin levels, the putative mechanism with regard to the causal relationship of genetic variation of NPFRR2 and obesity as mediated by individual's serum vitamin D status, is worth investigating in future studies.

Other gene targets identified were found in gene sets and pathways mostly independent from the canonical vitamin D synthesis and metabolism pathways. This is similar to the discoveries of Manousaki and Revez et al., in 2020, where both have established new and novel biological pathways outside the vitamin D pathways, that influence 25(OH)D levels. This discovery further demonstrated that the 25(OH) D metabolite is moderately polygenic. Identification of genetic regions not directly involved in 25(OH)D biology and related to environmental confounders that influence 25(OH)D levels has provided a deeper understanding of the genetic determinants contributing to variation in circulating vitamin D levels that may allow better genomic prediction of vitamin D levels and provide insights into biological mechanisms,[27] especially that two of the discovered genetic variants, namely the *KNG1* (rs11924390) and *SH2B1* (rs4788102), have been previously isolated and identified to be present among Filipino population cohort.[16,21]

A significant limitation continues to center on the interpretation and parsing of detected variants based on clinical utility. However, as comprehensive genomic variation in vitamin D metabolism continues to be documented, greater insight into the spectrum of variants underlying the phenotypic heterogeneity commonly observed in vitamin D deficiency subtypes has been gained. Although the sample sizes are the largest to date, they are small, especially for the Filipino population cohort (N=833).[29] Therefore, our results require replications in other vitamin D Filipino cohorts. We did not evaluate possible associations between SNP genotypes and vitamin D metabolite ratios, which may be considered as a further limitation. Furthermore, results of this study are not fully maximized because data on amount of exposure to sunlight, including time of day and duration of exposure were not collected. Data on vitamin D intake was not calculated because there is no vitamin D in the Philippine Food Composition Table at the time the survey was completed. Other factors such as use of sunblock, occupation and supplements were not interpreted in this paper because a previous study showed that these factors were not strong predictors of vitamin D status among Filipinos unlike gender, age and location.[30] Finally, in the absence of clear evidence of epistasis (i.e., the interaction between two or more genes to control a single phenotype), our findings suggest that a number of genetic and nongenetic risk factors (sex) may independently (and additively) contribute to the risk of vitamin D deficiency in this group of Filipino study participants of NNS.

There are some major advantages to restricting the genetic analysis to a limited set of genes: targeted enrichment and subsequent resequencing provides a superior quality of representation and a much higher read depth than whole genome sequencing. The result is promising that high accuracy could be obtained if the read depth is >200 coverage. It will provide a useful guidance for the future local genotyping studies. Moreover, clinical implementation of targeted Ampliseq® NGS has the potential to diagnose individuals with vitamin D deficiency with a high degree of speed and accuracy and at lower cost than either Sanger sequencing or whole exome sequencing. Secondly, most genetic studies of vitamin D status have been performed in European white descent cohorts. VDD does not appear exclusively in white individuals, and studies limited to a single race could miss VDD risk loci unique to different ancestries, at least among South East Asian-descendants.

Finally, to the best of our knowledge, this is the first Philippine pilot study on vitamin D status that encompasses all the available genetic evidence associated with non-communicable diseases, such as obesity and diabetes, given that previous work has been limited to describe a few genes involved in vitamin D pathways or the common genes identified by GWAS.[12,25,26]

An improved understanding of the genetic determinants of 25(OH)D will contribute in precise determination of the role of vitamin D in the etiology of complex diseases, such as musculoskeletal disorders, autoimmune disease, including multiple sclerosis, type 2 diabetes mellitus, obesity and even cancer. For example, four separate gene-disease studies have supported a protective effect of vitamin D against multiple sclerosis, and these results had clinical implications, reflected in recent clinical care guidelines for the use of vitamin D in preventing multiple sclerosis in those at risk, published by the MS Society of Canada. Moreover, in view of the important role in vitamin D metabolism of the genes identified, consideration should be given to develop a genetic panel that could be used in the clinical setting for those individuals or populations particularly vulnerable to vitamin D deficiency. In addition, the panel would be beneficial for patients with proven VDD or insufficiency who do not respond to appropriate doses of vitamin D supplementation, subjects who are vitamin D deficient in spite of enough sunlight exposure or who live in countries at an adequate latitude, and those with sufficient ingestion of vitamin D rich foods.

CONCLUSION AND RECOMMENDATION

Large-scale analysis of genes associated with lifestyle disease and other determinants of overall health have shown great utility in the discovery of genes and polymorphisms that play a role in vitamin D nutrition. Our data discovered at least six genetic variations that showed a statistically significant difference in serum vitamin D concentration across genotypes. These genes were previously shown to have contributed to disease pathologies such as type 2 diabetes mellitus, obesity, osteoporosis, and stress response. Although the exact molecular mechanisms of the discovered nutrigenetic markers remain to be determined, our study provides evidence that polymorphisms in lifestyle related genes play a role in vitamin D deficiency susceptibility. Identifying

genetic variants affecting functional status of vitamin D is important for understanding the role of this regulatory hormone in the prevention of certain diet related diseases such as type 2 diabetes mellitus, obesity, osteoporosis, and micronutrient deficiency and imbalance.

Taken together, this pilot study has provided additional evidence-based information on the putative contribution of genetic variants to optimizing intake recommendations, focusing on the Filipino population. There are many risk factors that have been reported to be associated with vitamin D deficiency, and genetic factors are among them. Further study is needed to explore the possibility of the involvement of genetic variations as risk factor for vitamin D deficiency and related diseases in the population. It is envisioned that understanding how genetic variations interact with environmental factors, especially nutrition, may hold the key to better prevention and management of diseases, particularly nutrition related diseases.

Moreover, data that may be generated from a national nutrition survey, where DOST-FNRI is the lead implementing agency, can provide an excellent opportunity to test the association of vitamin D deficiency with multiple health outcomes in a large, admixed sample of the general population.

The correction of low vitamin D concentrations can happen only if some or all of the following are implemented: the encouragement of safe, moderate exposure of skin to ultraviolet light; appropriate increases in food fortification with vitamin D; and the provision of vitamin D in diet to the *right person-individual and patient*, at the *right dose*, at the *right time.*

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ORIGINAL ARTICLE

Added Value of Postoperative Radioiodine Scan for Staging and Risk Stratification in Papillary Thyroid Microcarcinoma

Tawika Kaewchur,[1] Sirianong Namwongprom,[1] Nipawan Waisayanand,[2] Waralee Pongwiwattanachai,[1] Molrudee Ekmahachai[1]

[1]*Nuclear Medicine Division, Department of Radiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*
[2]*Endocrinology Division, Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*

Abstract

Objective. The complete staging and risk stratification of Papillary thyroid microcarcinoma (PTMC) is usually not done due to its theoretically low recurrence rates. This study aimed to determine the value of postoperative radioiodine diagnostic scan and SPECT/CT for the accurate staging and risk stratification in PTMC patients.

Methodology. This study was a retrospective review of PTMC patients from January 2014 to May 2017 who underwent I-131 scans. All PTMC patients were initially staged by the 8th edition AJCC/TNM staging system and risk-stratified, based on clinical information, histopathology and stimulated thyroglobulin (sTg). After I-131 scan, staging and risk stratification were re-assessed. The proportion of patients who ended up with a higher stage and risk stratification were reported.

Results and Conclusion. Fifty-two patients were included. The overall upgrading of cancer stage was 7.7 %. The overall higher risk stratification was 19.2% with radioiodine-avid lymph node, lung, and bone metastases. Neck and paratracheal node metastases were found in 37.3% of the initial low-risk patients with sTg less than 5 ng/mL. Lung metastasis was found in the initial intermediate-risk patient. The I-131 scan helps to localize metastatic lesions and results in a higher stage in 50% of the initial high-risk patients. This study provides some evidence showing the value of postoperative radioiodine WBS for accurate staging and risk stratification in PTMC patients. Larger studies with analytical design should be further performed to prove its significant utility.

Key words: papillary thyroid microcarcinoma, postoperative radioiodine scan

INTRODUCTION

Papillary thyroid microcarcinoma (PTMC) is a differentiated cancer of the thyroid gland in which tumor size does not exceed 1 cm in maximum diameter.[1] The incidence of PTMC has increased over the last 20 years.[2-4] PTMC without extrathyroidal extension or lymph node metastasis has an excellent prognosis with a low recurrent rate (less than 2%) and mortality rate (less than 1%).[5,6] Although PTMC has a low risk of recurrence, the previous retrospective studies revealed that cervical lymph node metastases and distant metastasis in PTMC were found 12.4-30% and 0.4%, respectively.[7,8]

For disease staging and risk stratification after surgery, postoperative serum-stimulated thyroglobulin (sTg), neck ultrasonography (US), and postoperative radioiodine whole-body scan (WBS) are commonly performed.[9,10] The sTg level has a high sensitivity to detect distant metastasis, but serum thyroglobulin antibody (Tg-Ab) can interfere with its measurement.[11] Neck US has a high sensitivity in detecting gross residual disease and cervical lymph node metastasis,[12] but it is unable to detect micrometastasis and, occasionally, is limited in distinguishing postoperative changes and residual disease. Postoperative radioiodine WBS has an added value in the staging by improving the detection of occult functional locoregional disease and distant metastasis, however, its benefit is still debatable for complete staging in differentiated thyroid cancer patients.[13-16]

Accurate staging and risk stratification are necessary for decision-making and guidance for proper subsequent I-131 treatment. Evidence of locoregional or distant metastasis strongly increases disease recurrence risk, leading to poorer disease-free survival.[17-19] This study aims to determine the incremental value of postoperative radioiodine scan and SPECT/CT for the accurate staging and risk stratification in PTMC patients.

METHODOLOGY

This retrospective study protocol was approved by the Research Ethics Committee of the Faculty of Medicine of Chiang Mai University. Informed consent was not required. We consecutively reviewed 343 patients with

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Corresponding author: Tawika Kaewchur, MD, FANMB
Nuclear Medicine Division, Department of Radiology
Faculty of Medicine, Chiang Mai University
110 Intawaroros Road, Sriphum, Muang Chiang Mai, Thailand 50200
Tel. No.: 6653935452
Fax No.: 6653936136
E-mail: tawika.k@cmu.ac.th
ORCiD: https://orcid.org/0000-0001-5309-8943

Table 1. Initial risk stratification categories[9,21]

Initial low risk	Initial intermediate risk	Initial high risk
No local or distant metastasis	Microscopic invasion to perithyroidal soft tissue	Known distant metastasis
Complete resection of macroscopic tumor	Presence of vascular invasion	Macroscopic tumor invasion to surrounding soft tissue
No evidence of locoregional invasion	Clinical or pathologic N1	Pathologic N1 with any metastatic lymph node size ≥3 cm in a greatest dimension
No vascular invasion	Multifocal PTMC with ETE	
Clinical N0 or pathologic N0		sTg >30 ng/mL

PTMC = papillary thyroid microcarcinoma; ETE = extrathyroid extention; sTg = stimulated thyroglobulin

pathologically proven differentiated thyroid cancer from January 2014 to May 2017 who underwent near-total or total thyroidectomy. During this period, 60 patients were identified with the PTMC diagnosis. PTMC is defined as thyroid cancer with a primary tumor size equal to or less than 1.0 cm.[1] All 60 patients underwent postoperative radioiodine WBS. Eight patients were excluded from this study due to the presence of Tg-Ab, which leads to unreliable sTg. Thus, the remaining 52 patients met the study criteria.

The study data included the patients' age and sex, surgical procedures, histopathologic results, postoperative sTg and postoperative I-131 whole-body scan findings. Postoperative sTg was measured on the day of I-131 ingestion by electrochemiluminescence immunoassay (ECLIA) technique, using the Cobas e411 system, with the functional sensitivity is at 0.09 ng/mL (measuring range 0.04-5,000 ng/mL).

Before imaging, the disease staging and risk stratification in each patient was initially determined following the staging system of the 8th edition AJCC/TNM Cancer Staging[20] and the 2015 American Thyroid Association (ATA) Guidelines,[9] based on clinical and pathologic data. In addition to the ATA guidelines, a high level of sTg was defined as higher than 30 ng/mL.[21] The initial risk categories were shown in Table 1.

For the imaging technique, postoperative radioiodine WBS with additional spot planar images of anterior neck and chest as well as both lateral view of the neck were performed at 48 hours after the ingestion of I-131 37 MBq (1 mCi). All patients were prepared by thyroid hormone withdrawal at least four weeks before imaging to elevate serum TSH greater than 30 uIU/mL and dietary iodine restriction for two weeks. The images were acquired using a dual-head gamma camera (Symbia T, Siemens, USA) with high energy general-purpose (HEGP) collimator, a 20% energy window centered on a 364 keV photopeak, and the scan speed of 8 cm/min. The patients who showed radioiodine avidity outside the thyroid bed also received additional hybrid single-photon emission computed tomography/computed tomography (SPECT/CT) to localize the lesion. SPECT images were acquired by 128 × 128 matrices over 360° with 32 views (45 secs/view, step and shoot technique) using a 364 keV photopeak and 20% energy window, followed by a low dose CT scan for anatomical localization on the same instrument. SPECT images were reconstructed with filtered back projection and fused with CT images.

Postoperative radioiodine WBS of each patient was interpreted by two nuclear medicine physicians (20-year and 5-year experienced) with blinded consensus. After imaging, disease staging and the risk stratification were subsequently re-assessed in each patient.

The review data were shown in mean ± standard deviation (SD), range, and percentage. After WBS, the percentage of staging and risk stratification change were analyzed.

RESULTS

Of the 52 PTMC patients, 42 patients (80.8%) were females and 10 patients (18.2%) were males. The mean age of all patients was 45.7±14.0 years (age range 13-69 years). Patients underwent near-total (7.7%) or total (92.3%) thyroidectomy due to treatment of large multinodular goiter (38.4%), presence of thyroid nodules in both lobes on the pre-operative US (21.2%), suspected thyroid capsule invasion on US (25%) and the suspected metastatic cervical node on the pre-operative US (15.4%). Sixteen patients (30.8%) had cervical node dissection due to the suspected metastasis on the pre-operative US (8 patients) and intraoperative finding (8 patients). Postoperative radioiodine WBS was performed with a mean interval of 6.5±1.36 weeks after the surgery. All patients showed no clinical symptoms of distant metastasis before surgery. Demographic, pathologic data, and sTg level were shown in Table 2. Of the 52 patients, results of WBS changed the disease staging in four patients (7.7%) (Table 3) and risk stratification in ten patients (19.2%) (Table 4).

For disease staging, 36 patients younger than 55-year-old were all in Stage I. After postoperative WBS was performed, one of these (2.8%) was upgraded to Stage II

Table 2. Demographic, pathologic data, and laboratory results

Demographic data	N (%)
Gender	
Female	42 (80.8%)
Male	10 (19.2%)
Age	
<55 years	36 (69.2%)
≥55 years	16 (30.8%)
Pathology	
Cell type	
Papillary with classic variant	32 (61.5%)
Papillary with follicular variant	20 (38.5%)
Multiple tumor foci	20 (38.5%)
Presence of lymphovascular invasion	13 (25.0%)
Surgical margin involvement	8 (15.3%)
Extrathyroidal extension	
Microscopic	4 (7.7%)
Macroscopic	0
Pathological lymph node metastasis	
Absence (N0)	6 (11.6%)
Presence (N1)	10 (19.2%)
No neck node dissection (Nx)	36 (69.2%)
Serum thyroglobulin level	
<2.0 ng/mL	31 (59.7%)
2.0 to <30 ng/mL	13 (25.0%)
≥30 ng/mL	8 (15.3%)

Nx = unknown nodal metastasis status

Table 3. Changes in disease staging (AJCC/TNM 8th Edition) with postoperative WBS results

	Initial staging N (%)		After WBS N (%)
<55 years old (N = 36)			
Stage I	36 (100%)	Stage I	35 (97.2%)
		Stage II	1 (2.8%, lung)
≥55 years old (N = 16)			
Stage I	12 (75.0%)	Stage I	10 (62.5%)
		Stage II	1 (6.25%, node)
		Stage IV	1 (6.25%, lung)
Stage II	4 (25.0%, node)	Stage II	3 (18.75%)
		Stage IV	1 (6.25%, bone)

WBS = whole body scan

Table 4. Changes in risk stratification for disease recurrence with postoperative WBS results

Initial ATA risk stratification N (%)		Risk stratification after WBS N (%)	
Initial low	24 (46.2%)	Low	15 (28.8%)
		Intermediate	9 (17.4%)
Initial intermediate	20 (38.5%)	Intermediate	19 (36.5%)
		High	1 (2.0%)
Initial high	8 (15.3%)	High	8 (15.3%)

WBS = whole body scan

due to radioiodine-avid lung metastasis. For 16 patients ≥55 years, three patients changed to a higher stage due to the lymph node, lung, and bone metastases (Table 3).

Of the 24 patients initially defined as low risk, nine patients (37.3%) were grouped to intermediate-risk due to neck node metastases detected by WBS. The metastatic lymph nodes were central in 6 patients (67%), lateral in 2 patients (22%), and supraclavicular in 1 patient (11%). All metastatic nodes were equal or less than one cm in size. None of these patients had neck node dissection, and their postoperative sTg levels were low (undetectable to 4.9 ng/mL). Figure 1 showed that the planar image of postoperative I-131 scan (Figure 1A) of an initial low-risk patient with undetectable stimulated thyroglobulin revealed subcentimeter radioiodine-avid right upper cervical and right supraclavicular (SPC) lymph node metastases as demonstrated on SPECT/CT image (Figure 1B and 1C).

Of the 20 patients initially defined as intermediate risk, one patient (5%) with sTg of 23.2 ng/mL was re-grouped to high risk according to bilateral lung metastases detected on WBS (Figure 2A and 2B), corresponding with multiple tiny lung metastases on CT images (Figure 2C and 2D). In another six patients (30%), which remained grouped as intermediate risk, radioiodine-avid lymph nodes were found. The metastatic lymph nodes were central neck in 2 patients (10%), lateral neck in 2 patients (10%), and paratracheal in 2 patients (10%) region. All metastatic nodes were equal or less than one cm in size.

All eight patients, who were initially defined as high risk, had a high sTg >30 ng/mL. None of these had macroscopic tumor invasion or large neck node metastasis (>3 cm). Postoperative WBS assisted with detecting lymph node metastasis in two patients (25%, lateral and paratracheal lymph nodes). Distant metastases were found in two patients, with one patient in the lungs (12.5%) and another one (12.5%) in multiple levels of the spine, sacrum, and left proximal femur. No radioiodine-avid cervical neck node or distant metastasis was demonstrated in the remaining four initial high-risk patients.

DISCUSSION

This study was performed to address the lack of clinical data regarding the clinical benefit of postoperative radioiodine WBS, which is not routinely performed in PTMC patients. In our study, WBS added the value of accurate staging and risk stratification by identifying radioiodine-avid lymph nodes and distant metastasis, which resulted in the required subsequent postoperative I-131 treatment. As we found subclinical regional lymph node and distant metastases by WBS, these PTMC patients had a potential for the disease recurrence. In our study, the change of the risk stratification of recurrence is more pronounced than that of the staging because the detection of unexpected lymph node metastasis did not change the staging or mortality in patients younger than 55 years.

The changes in staging and risk stratification in PTMC patients in our study were concordant with prior studies[13,22-25] that showed the benefits of WBS by changing the staging and the recurrent risk in overall DTC patients. For the curative intent, the WBS findings can improve disease-free mortality rate and recurrence rate by identifying unexpected regional lymph node or distant metastasis leading to higher dose I-131 treatment with 5,550–7,400 MBq (150-200 mCi). Low-risk patients without aggressive features or metastasis are not routinely recommended for radioiodine ablation with 1,110 MBq (30 mCi).[9,26] Detection of metastasis also impacts the selection of potential surgical candidates in the case who presented with large metastatic lesions before I-131 treatment to improve treatment outcomes and optimize long-term follow-up.

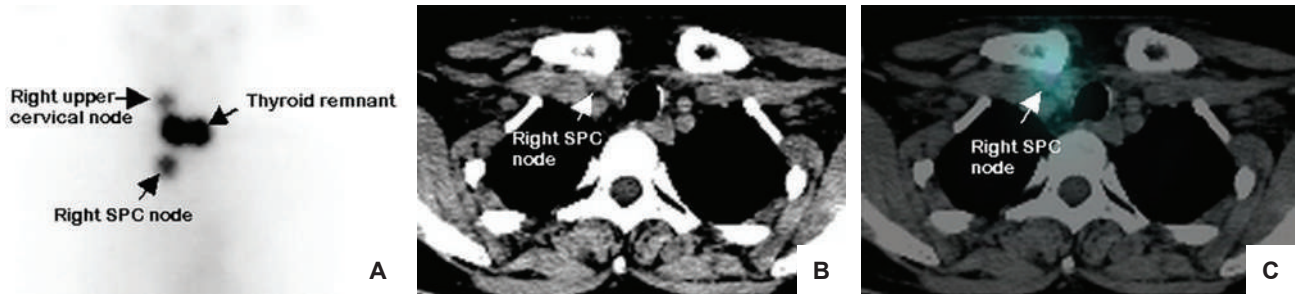

Figure 1. (A) Postoperative I-131 scan revealed two radioiodine-avid right upper cervical and supraclavicular (SPC) node metastases. The right SPC node was demonstrated in these **(B)** CT scan and **(C)** SPECT images.

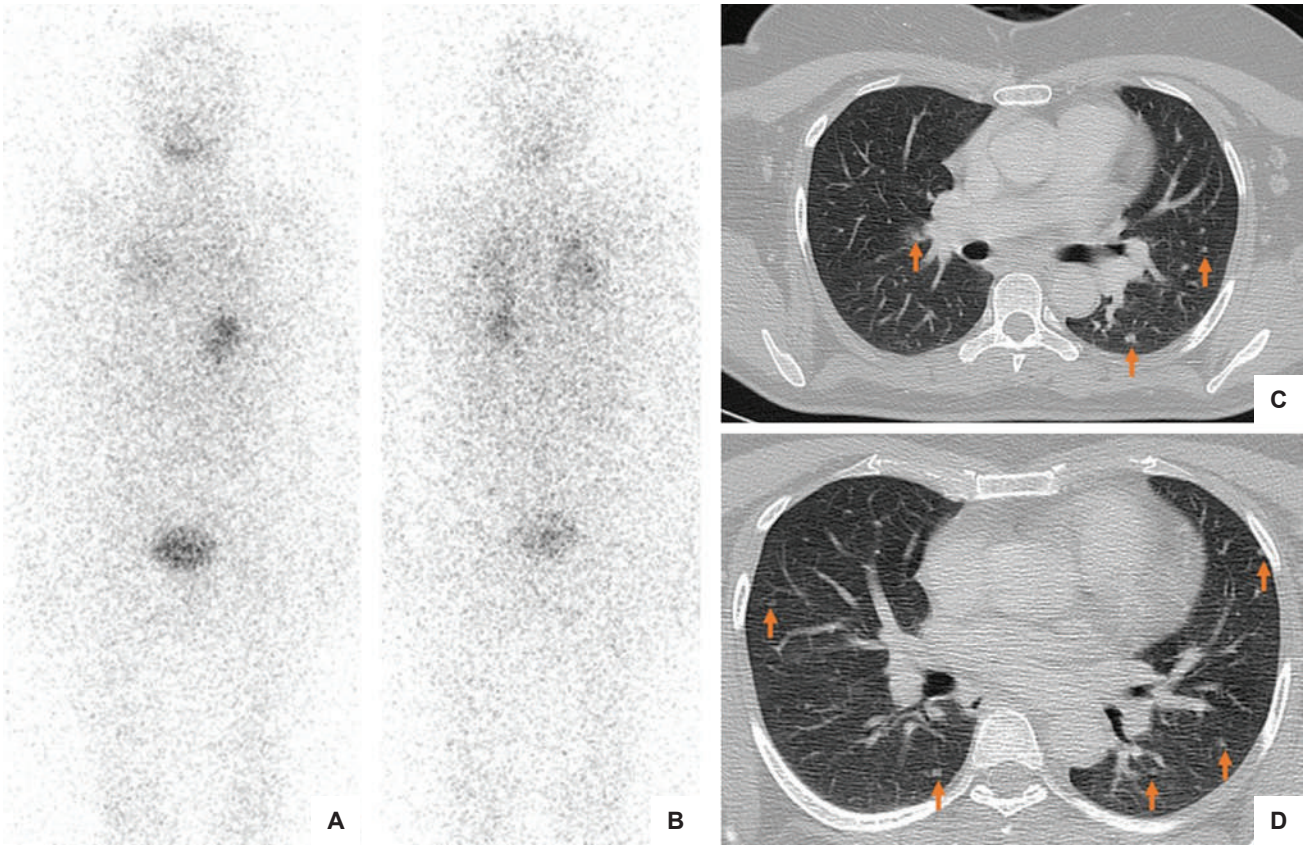

Figure 2. (A,B) Post-operative I-131 scan reveals diffuse radioiodine uptake in the bilateral lungs, corresponding with multiple tiny pulmonary metastases as seen on **(C,D)** CT images (orange arrows).

Radioiodine WBS helps demonstrate occult lymph node metastasis, mainly in the central compartment, that is difficult to detect by other investigation methods. Moreover, sTg in these patients (<5 ng/mL) did not show any clue of metastasis. These metastatic patients with low measurable sTg are possibly due to too small tumor volume to synthesize Tg and partial loss of Tg secretory function from the tumor cells into the blood.[27] Thus, complementary imaging is necessary for long-term follow-up or detecting suspected recurrence in these undetectable sTg patients. The neck US has a high specificity to detect cervical lymph node metastasis, however, the small size of the central lymph node is difficult to detect by US.[28,29] Among the high-risk patients, who had high sTg levels suspected for distant metastasis, radioiodine WBS demonstrates metastatic location in about 50% of the cases in our study. In undetectable metastasis cases by WBS, re-evaluation on post-treatment I-131 WBS and follow-up sTg is necessary. Correlative anatomical imaging will be considered in the patients with persistent or progressively rising sTg levels without radioiodine-avid metastasis, and these patients are unlikely to respond to I-131 treatment.

Our study had some limitations. First, a small sample size as we focused on PTMC patients who generally had a good prognosis. Second, as a retrospective study, there might be some bias in clinical profile or investigations leading to treating these PTMC patients with near-total or total thyroidectomy, which guidelines recommend mostly can be treated by lobectomy.

CONCLUSION

The study provides some evidence showing the value of postoperative radioiodine WBS for accurate staging and recurrent risk stratification by detecting metastatic lesions in PTMC patients, particularly those with initial low risk with low sTg. Larger studies that are able to test for statistical significance should be done to further prove its added utility in the diagnosis and management of papillary thyroid microcarcinoma.

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ORIGINAL ARTICLE

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Comparison of the Diagnostic Performance of Ultrasound-Based Thyroid Imaging Reporting and Data System (TIRADS) Classification with American Thyroid Association (ATA) Guidelines in the Prediction of Thyroid Malignancy in a Single Tertiary Center in Manila, Philippines

Ryan James Gacayan,[1] Ruben Kasala,[1] Ma. Patricia Puno-Ramos,[1] Dondee Jules Mojica,[2] Ma. Krisha Castro[2]

[1]*Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, The Medical City, Philippines*
[2]*Department of Radiology, The Medical City, Philippines*

Abstract

Objective. To compare the diagnostic performance of American College of Radiology-Thyroid Image Reporting and Data Systems (ACR-TIRADS) and the American Thyroid Association (ATA) guidelines on screening for thyroid malignancy.

Methodology. A cross-sectional criterion-referenced study involving Filipino patients with thyroid nodules, 18-80 years old, who underwent ultrasound guided fine needle aspiration biopsy at the Thyroid Clinic of The Medical City from July to December 2019. The ACR-TIRADS and the ATA guidelines were compared for 197 nodules. Standard diagnostic parameters were calculated, namely sensitivity, specificity, PPV, NPV, positive and negative likelihood ratios and overall accuracies.

Results. The risks of malignancy were 15% and 22% for TIRADS 4 and 5 respectively. For ATA guidelines, it's 2%, 20%, and 15% for nodules with low, intermediate, and high suspicion respectively. The sensitivity, specificity, PPV, NPV, and accuracy of the American College of Radiology Thyroid Imaging Reporting and Data System (ACR TIRADS) in relation to Fine Needle Aspiration Cytology (FNAC) is 100%, 52.2%, 16.5%, 100%, and 56.4% respectively. For the American Thyroid Association (ATA) guidelines it is 88.2%, 57.8%, 16.5%, 98.1%, and 60.4% respectively.

Conclusion. The ACR TIRADS classifications appears to be more sensitive than the ATA classification. The ATA guidelines prove to be a more specific test. Each tool has its unique advantages and disadvantages. Therefore, clinicians must use these tools with utmost vigilance to avoid over or under diagnosis and to avoid unnecessary thyroid nodule biopsies.

Key words: Thyroid Imaging Reporting and Data System, American Thyroid Association, thyroid cancer, malignancy risk, thyroid nodules, ultrasound of thyroid

INTRODUCTION

Depending on the study population, the prevalence of thyroid nodules ranges from as low as 2% to as high as 35%.[1,2] In a 2012 nationwide study from Carlos-Raboca et al.,[3] involving 4,897 subjects, the estimated prevalence of nodular goiter in the Philippines is 8.9%.

The American Thyroid Association lists these sonographic findings suggestive of malignancy: solid nodules, nodule hypoechogenicity or marked hypoechogenicity, irregular margins, microcalcifications and a shape taller than wide on a transverse view. The varied spectrum of sizes and characteristics of thyroid nodules makes it difficult to select which nodule is a candidate for fine needle aspiration biopsy (FNAB).[4] Fine needle aspiration biopsy is a minimally invasive diagnostic procedure with published sensitivity and specificity that ranges between 65% to 98% and 73% to 100%, respectively.[5] The accuracy of ultrasound guided FNAB in different studies, both locally and abroad may range from 77.3% to as high 96.7%.[5,6] According to Cibas et al., only 3% to 7% of FNA cytology are malignant and most nodules are benign.[7] Given this, it is important to use an ultrasound classification that will help differentiate benign from malignant thyroid nodules to determine which nodule(s) will require FNAB and decrease unnecessary procedures.

Kwak et al., sought to implement a similar standardized model for thyroid nodules with the release of the Thyroid Imaging Reporting and Data System (TIRADS) in 2011.[8] In addition, in order to avoid the over usage of FNA for multiple benign thyroid nodules, several reports investigated the risk of malignant nodules for ultrasound-guided biopsy due to suspicious ultrasonographic features.[9-10] Park et al.,[11] and Horvath et al.,[12] established

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Corresponding author: Ryan James B. Gacayan, MD
Section of Endocrinology, Diabetes and Metabolism
Department of Medicine, The Medical City
Ortigas Avenue, Pasig City, Metro Manila, Philippines
Tel. No. +632-8988-1000
E-mail: ryan_james8423@yahoo.com
ORCiD: https://orcid.org/0000-0002-9932-2602

a thyroid ultrasonographic system to stratify cancer risk and developed several categories based on 10 and 12 sonographic features, called the thyroid imaging reporting and data system (TIRADS).

An updated version endorsed by the American College of Radiology (ACR) was released in 2017.[8,12] This does not include subcategories, nor does it include TIRADS 0 category which indicates a normal thyroid gland.[13] TIRADS categories range from TIRADS 1 to TIRADS 5.

A retrospective study by Middleton et al., comparing TIRADS with ATA and other scoring system showed that 13.9% of nodules could not be categorized using ATA guidelines and 9.4% of these non-categorized nodules were malignant.[14] The committee of ACR-TIRADS decided against the pattern-based approach used by ATA based on the results of a study by Yoon et al., which showed that using ATA guidelines, they were unable to classify 3.4% of 1,293 nodules, of which 18.2% were malignant. In this study, they only included nodules that were subjected to FNA or surgery.[8]

This study follows on the findings of a retrospective study done at The Medical City, Manila, Philippines by Dy and Kasala et al. The authors recommended a multicenter prospective study for the use of TIRADS as their research concluded that TIRADS was sensitive in recognizing patients with thyroid cancer and can be used as a guide in deciding the need for fine needle aspiration biopsy.[15]

GENERAL OBJECTIVES

To compare the diagnostic performance of Thyroid Imaging reporting and data System (TIRADS) and the American Thyroid Association (ATA) guidelines on screening for thyroid malignancy.

SPECIFIC OBJECTIVES

To determine the diagnostic performance of TIRADS in screening for thyroid malignancy in terms of:

a. Sensitivity and specificity
b. Positive Predictive Value and Negative Predictive Value
c. Positive Likelihood Ratio and Negative Likelihood Ratio
d. Diagnostic accuracy

To determine the diagnostic performance of ATA in screening for thyroid malignancy in terms of:

a. Sensitivity and specificity
b. Positive Predictive Value and Negative Predictive Value
c. Positive Likelihood Ratio and Negative Likelihood Ratio
d. Diagnostic accuracy

METHODOLOGY

Study design

This is a cross-sectional criterion-referenced study approved by our institutional review board. During the time frame specified, all patients who were undergoing ultrasound-guided FNA were asked to participate in the study with a signed informed consent.

Study population

This study enrolled Filipino patients with thyroid nodules aging 18 to 80 years old who underwent ultrasound guided fine needle aspiration biopsy of thyroid nodules at the Thyroid Clinic of The Medical City from July 2019 to December 2019. Patients are excluded if the cytology report of the FNAB is inadequate or non-diagnostic.

Sample size

A minimum of 90 patients were required for this study based on a level of significance of 5%, a prevalence of 33.56%, sensitivity of 98% with a marginal error of 0.05. The values for the prevalence of thyroid malignancy and sensitivity of TIRADS were based from the study by Dy and Kasala et al.[15]

Description of study procedure

All qualified subjects underwent a repeat ultrasound of thyroid gland using BK Flex Focus 800 ultrasound machine prior to their scheduled ultrasound guided fine needle aspiration biopsy. The scanning protocol in our study includes scanning of thyroid gland and cervical lymph nodes in both transverse and longitudinal planes by B–mode (brightness mode), CCDI (Color-coded Doppler imaging) and PDI (Power Doppler imaging). The ultrasonography of the thyroid gland was done by a second-year radiology resident. It was then reviewed and read by only 1 radiologist with more than ten years experience. The nodules were analyzed according to their type (solid, cystic, or mixed), echogenicity, margins, shape, echogenic foci, and evidence of calcification. The reports were categorized into two, ACR TIRADS and conventional ATA guidelines respectively.

American College of Radiology – Thyroid Imaging, Reporting and Data System (ACR TIRADS) described nodules according to composition, echogenicity, shape, margin, and echogenic foci and a corresponding point or points will be given. Points were added from all categories to determine the TIRADS level and nodules were classified into the following: TIRADS 1 benign, TIRADS 2 not suspicious, TIRADS 3 mildly suspicious, TIRADS 4 moderately suspicious, and TIRADS 5 highly suspicious (Appendix A).

A second report was provided and the nodules were described according to its size, location, composition (solid, cystic proportion, or spongiform), echogenicity, margins, presence and type of calcifications, and shape if taller than wide, and vascularity. The nodules were re-classified according to American Thyroid Association (ATA) Guidelines 2015 into the following based on the sonographic pattern: benign, very low suspicion, low suspicion, intermediate suspicion, and high suspicion (Appendix B).

The cytology reports were used to classify nodules into five categories using Bethesda Classification: I for non-diagnostic, II for benign, III for atypia of undetermined significance, IV for follicular neoplasm or suspicious for follicular neoplasm, V suspicious for malignancy, VI for malignant. Nodules with FNA results that were classified as Bethesda II to VI were considered diagnostic and included in the final analysis. The nodules classified as Bethesda cytology IV, V, and VI were considered as suspicious for malignancy and Bethesda cytology II and III were benign.

All other nodules were excluded unless the nodule was resected and histologic findings were available.

Statistical analysis

Descriptive statistics were used to summarize the data: frequency and proportion for nominal variables; median (range) and mean ± standard deviation (SD) for interval/ratio variables with and without normal distributions, respectively. Test on proportions was used to determine differences in proportions of nodules recommended for FNA between TIRADS and ATA.

Standard diagnostic parameters were calculated for the two sonographic criteria, namely sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive and negative likelihood ratios (LR), all with their corresponding 95% confidence intervals (CI). McNemar's test was used to compare overall accuracies of TIRADS and ATA recommendations for FNA. All valid data were included in the analysis. Missing variables were neither imputed nor estimated. Null hypotheses were rejected at 0.05 α-level of significance. STATA 15.0 was used for data analysis.

RESULTS

A total of 197 nodules from 121 patients (Figure 1), with median age of 53 (21–77) years and comprised mostly of females (85%), were included in the analysis (Table 1). Thyroid nodules were located almost equally on either side. Sonographically, half of the lesions measured 1.0–1.9 cm, 81% were solid or almost completely solid, 70% were hyperechoic or isoechoic, 88% were wider-than-tall, 92% possessed smooth margins, and 67% contained no echogenic focus or only a large comet-tail artifact.

Most patients were classified as moderately (41%) or mildly (35%) suspicious for malignancy by TIRADS classification. By ATA guidelines, 43% and 31% were of low and high suspicion, respectively. Cytologic analysis revealed most nodules (83%) to be benign, and only 8% were suspicious or obviously malignant. Of the latter nodules, 40% turned out benign on surgical histopathology.

The risks of malignancy were 15% for nodules considered moderately suspicious (TIRADS 4) and 22% for those that were highly suspicious (TIRADS 5). No malignant diagnoses were made among those with lower grade classification (TIRADS 1,2, and 3). The largest mean (± SD) nodular size was with mildly suspicious lesions, at 2.3±1.0 cm (Table 2).

On the other hand, the risks of malignancy were 2%, 20%, and 15% for nodules at low, intermediate, and high suspicion by ATA guidelines (Table 3). There were no malignant findings among the lower grade lesions. The largest lesion sizes were with low (2.3±1.0 cm) and high (2.2±1.1 cm) suspicion.

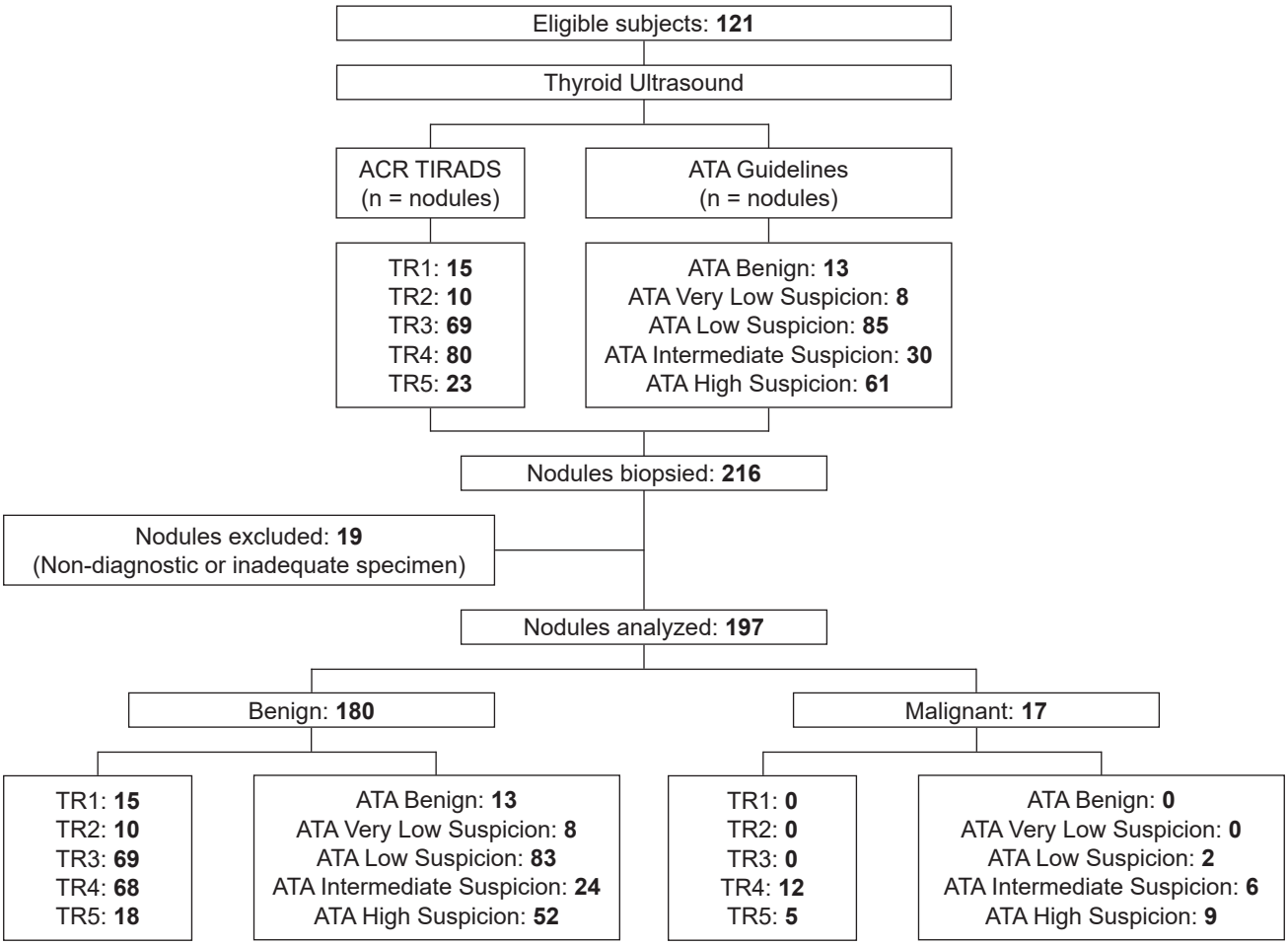

Figure 1. Flow chart of patients in the study. *TR – TIRADS, ATA – American Thyroid Association*

Table 1. Patient and nodule characteristics (n=197 nodules, 121 patients)

	Median (Range); Count (%)
Age (years)	53 (21–77)
Sex	
Male	18 (14.88)
Female	103 (85.12)
Solitary nodule	59 (48.76)
Nodule (cm)	
0–0.4	0
0.5–0.9	11 (5.58)
1.0–1.4	51 (25.89)
1.5–1.9	48 (24.37)
2.0–2.4	35 (17.77)
2.5–2.9	13 (6.60)
≥ 3.0	39 (19.80)
Location	
Left	96 (48.73)
Right	97 (49.24)
Isthmus	4 (2.03)
Composition	
Cystic	1 (0.51)
Almost completely cystic	10 (5.08)
Spongiform	4 (2.03)
Mixed cystic & solid	23 (11.68)
Solid or almost completely solid	159 (80.71)
Echogenicity	
Anechoic	14 (7.11)
Hyperechoic or isoechoic	138 (70.05)
Hypoechoic	40 (20.30)
Very Hypoechoic	5 (2.54)
Shape	
Wider-than-tall	174 (88.32)
Taller-than-wide	23 (11.68)
Margin	
Smooth	181 (91.88)
Ill-defined	11 (5.58)
Lobulated or irregular	5 (2.54)
Extra-thyroidal extension	0
Echogenic foci	
None or large comet-tail artifact	132 (67.01)
Macrocalcification	26 (13.20)
Peripheral or rim calcifications	9 (4.57)
Punctate echogenic foci	30 (15.23)
ACR TIRADS category	
Benign	15 (7.61)
Not suspicious	10 (5.08)
Mildly suspicious	69 (35.03)
Moderately suspicious	80 (40.61)
Highly suspicious	23 (11.68)
ATA classification	
Benign	13 (6.60)
Very low suspicion	8 (4.06)
Low suspicion	85 (43.15)
Intermediate suspicion	30 (15.23)
High suspicion	61 (30.96)
Bethesda system[a]	
I	0
II	164 (83.25)
III	16 (8.12)
IV	1 (0.51)
V	11 (5.58)
VI	5 (2.54)
Histopathology (n=15)	
Papillary thyroid carcinoma	8 (53.33)
Multinodular colloid goiter	6 (40.00)
Follicular thyroid carcinoma	1 (6.67)

[a] Bethesda system: I, non-diagnostic or unsatisfactory; II, benign; III, atypia of undetermined significance or follicular lesion of undetermined significance; IV, follicular neoplasm or suspicious for it; V, suspicious for malignancy; VI, malignant.

Table 2. Nodule size and risk of malignancy, by TIRADS category (n=197)

TIRADS	FNAC		Risk of Malignancy (%)	Nodule Size (cm)
	Suspicious for Malignancy (n=17)	Benign (n=180)		
Benign (TR1)	0/15	15/15	0	1.73 ± 0.60
Not suspicious (TR2)	0/10	10/10	0	1.91 ± 0.47
Mildly suspicious (TR3)	0/69	69/69	0	2.30 ± 1.03
Moderately suspicious (TR4)	12/80	68/80	15.00	2.06 ± 0.98
Highly suspicious (TR5)	5/23	18/23	21.74	1.94 ± 1.35

Table 3. Nodule size and risk of malignancy, by ATA classification (n=197)

ATA	FNAC		Risk of Malignancy (%)	Nodule Size (cm)
	Suspicious for Malignancy (n=17)	Benign (n=180)		
Benign	0/13	13/13	0	1.82 ± 0.59
Very low suspicion	0/8	8/8	0	1.77 ± 0.49
Low suspicion	2/85	83/85	2.35	2.30 ± 1.02
Intermediate suspicion	6/30	24/30	20.00	1.52 ± 0.71
High suspicion	9/61	52/61	14.75	2.20 ± 1.13

Using TIRADS

Using FNAC as the gold standard, TIRADS had a high sensitivity, with 100% (17/17) of the nodules that were suspicious for malignancy by FNAB having positive finding (IV-V). However, its ability to correctly rule out benign nodules was quite low, with only 52.2% of those who had negative findings (I-III) having benign nodules (specificity) (Table 4).

Positive findings in the TIRADS were about 2.09 times as likely to be observed in malignant nodules as compared to benign nodules (LR+). Negative findings were about 100% less likely to be seen in malignant nodules as opposed to benign nodules (LR-) (Table 4).

Using TIRADS, the probability that positive findings are malignant in the FNAC is 16.5% (PPV), whereas negative findings have 100% chance of having benign results (NPV) (Table 4).

Using ATA

ATA compared to TIRADS had a lower sensitivity with 88.2% (15/17) of nodules that were suspicious for malignancy by FNAB had a positive ATA category of IV-V. The system was unsatisfactory in correctly ruling out benign nodules, with only 57.8% of those who had negative findings (category I-III) having benign nodules (specificity) (Table 5).

Positive findings in the ATA were about 2.09 times as likely to be observed in malignant nodules as compared to benign nodules (LR+). Negative findings were about 80% less likely to be seen in malignant nodules as opposed to benign nodules (LR-) (Table 5).

Table 4. Accuracy of FNA recommendation to detect malignancy using TIRADS

TIRADS	Malignant (FNAC+)	Benign (FNAC-)	Total
	Frequency (%)		
TIRADS IV-V	17 (100)	86 (47.78)	103 (52.28)
TIRADS I-III	0	94 (52.22)	94 (47.72)
Total	17 (8.63)	180 (91.37)	197
Sensitivity		100% (80.5 to 100)	
Specificity		52.2% (44.7 to 59.7)	
Positive Predictive Value (PPV)		16.5% (14.5 to 18.7)	
Negative Predictive Value (NPV)		100%	
Positive Likelihood Ratio		2.09 (1.80 to 2.44)	
Negative Likelihood Ratio		0	
Accuracy		56.4% (49.1 to 63.4)	

Table 5. Accuracy of FNA recommendation to detect malignancy using ATA

ATA	Malignant (FNAC+)	Benign (FNAC-)	Total
	Frequency (%)		
ATA IV-V	15 (88.24)	76 (42.22)	91 (46.19)
ATA I-III	2 (11.76)	104 (57.78)	106 (53.81)
Total	17 (8.63)	180 (91.37)	197
Sensitivity		88.2% (63.6 to 98.5)	
Specificity		57.8% (50.2 to 65.1)	
Positive Predictive Value (PPV)		16.5% (13.4 to 20.1)	
Negative Predictive Value (NPV)		98.1% (93.4 to 99.4)	
Positive Likelihood Ratio		2.09 (1.64 to 2.67)	
Negative Likelihood Ratio		0.20 (0.06 to 0.75)	
Accuracy		60.4% (53.2 to 67.3)	

Table 6. Summary of diagnostic performance of TIRADS and ATA

	ATA	TIRADS
Sensitivity (%)	88.2 (63.6 to 98.5)	100% (80.5 to 100)
Specificity (%)	57.8% (50.2 to 65.1)	52.2% (44.7 to 59.7)
PPV (%)	16.5% (13.4 to 20.1)	16.5% (14.5 to 18.7)
NPV (%)	98.1% (93.4 to 99.4)	100%
Positive LR	2.09 (1.64 to 2.67)	2.09 (1.80 to 2.44)
Negative LR	0.20 (0.06 to 0.75)	0
Accuracy (%)	60.4% (53.2 to 67.3)	56.4% (49.1 to 63.4)
McNemar's test p-value	0.004	

Table 6.1. Comparison of ATA and TIRADS

	TIRADS		ATA	
	I-III	IV-V	I-III	IV-V
	Frequency (%)			
All nodules (n=197)				
Malignant nodules based on FNAC (n=17)	0	17 (100)	2 (11.76)	15 (88.24)
Benign nodules based on FNAC (n=180)	94 (52.22)	86 (47.78)	104 (57.78)	76 (42.22)
Solitary nodules (n=59)				
Malignant solitary nodules (n=6)	0	6 (100)	1 (16.67)	5 (83.33)
Benign solitary nodules (n=53)	17 (32.08)	36 (67.92)	21 (39.62)	32 (60.38)

Using ATA, the probability that negative findings are malignant in the FNAC is 16.5% (PPV), whereas positive findings have 98.1% chance of having benign results (NPV) (Table 5).

Overall accuracies of the FNA recommendation by TIRADS and ATA criteria were moderate (56.4% [95% CI 49.1–63.4) and 60.4% [95% CI 53.2–67.3], *P*=0.004). The former's sensitivity was high at 100% (95% CI 80.5–100), but the latter was inferior at 88.2% (95% CI 63.6–98.5). Both sonographic criteria had NPV's above 95% (Table 6).

DISCUSSION

The pathological nature of thyroid nodules directly affects the therapeutic decisions and patient prognosis; therefore, the correct diagnosis of thyroid nodules at an early stage has important clinical significance. However, conventional sonographic diagnoses for thyroid nodules presents limitations related to overlapping boundaries, morphologies, internal blood streams, and echoes between malignant and benign nodules. In addition, subjective factors related to the diagnostician can also affect the accuracy of the diagnosis. Therefore, research by Kwak,[8] Park[11] and Horvath[12] indicates that the thyroid imaging reporting and data system (TIRADS) can be used to improve the diagnostic accuracy of thyroid nodules by ultrasound, which will provide improvements that can be used in clinical practice. This study was done to compare the diagnostic performance of ACR TIRADS and ATA guidelines for predicting risk of thyroid malignancy.

The suggested risk of malignancy for TIRADS is less than 2% for TIRADS 1 and TIRADS 2, 5% for TIRADS 3, 5-20% in TIRADS 4, and greater than 20% for TIRADS 5.[16] In our study, the risk of malignancy was 15% for nodules considered moderately suspicious or TIRADS 4 and 22% for those that were highly suspicious or TIRADS 5 which are well matched to the suggested risk of malignancy by ACR TIRADS. When compared to another local study done by Dy and Kasala et al.,[15] the malignancy risk for TIRADS 4 was 12.82% to 53% and is well matched with our result. The malignancy risk for TIRADS 5 in the former study was 66.67% which is higher than our result.[15] Selection bias may have contributed to the very high malignancy risk since it was a retrospective study.

The risk of malignancy recommended by the ATA is more than 70-90% for the high suspicion pattern, 10-20% for the intermediate suspicion pattern, 5-10% for the low suspicion pattern, less than 3% for the very low suspicion pattern and less than 1% for the benign pattern.[4] The risk of malignancy was 2%, 20%, and 15% for nodules at low, intermediate, and high suspicion respectively in our study by ATA guidelines. Only intermediate suspicion nodules matched the suggested risk of malignancy by ATA guidelines.

The diagnostic performance of both ACR TIRADS and the ATA guidelines are one of the most commonly compared sonographic classification of nodules in various studies. They both have outstanding performances with sensitivity ranging from 70% to 90% and specificity of 33% to 67%.[16] These are international studies and most of them are retrospective in nature.

In a local retrospective study by Dy and Kasala et al., TIRADS classification for predicting thyroid malignancy still maintained a very high sensitivity of 98%. However,

specificity is quite low at 7.07% which is also lower compared to other studies.[15] In our study, ACR TIRADS had a high sensitivity of 100% which is higher than Dy and Kasala et al., (98%)[15] Horvath et al., (88%)[11] Ha et al., (74.7%)[16] and Grani et al. (83%).[17]

On the other hand, ACR TIRADS in our study had a specificity of 52.2% which is higher than Dy and Kasala et al., (7.07%)[15] and Horvath et al., (49%)[11] but slightly lower than Grani et al., (56.2%)[17] and Ha et al., (67.3%).[16] The overall accuracy of ACR TIRADS is 56.4% which is higher than Dy and Kasala et al., (53%)[15] but lower than Ha et al., (69%)[16] and Horvath et al., (94%).[11] The low accuracy of ACR TIRADS in our study is probably due to high false positive rate (48%).

The prevalence of malignancy in our study is 8.63% which is similar to the malignancy rate according to Cibas et al.,[7] at 3% to 7%. When using ACR TIRADS, the probability that FNA recommended nodules are malignant in the FNAC is 16.5% (PPV) which is almost similar to Grani et al., (12.8%)[17] but lower than Ha et al,. (40.2%)[16] Horvath et al., (49%)[11] and Dy and Kasala et al., (34.75%)[15]. Thyroid nodules that are not recommended for biopsy when using ACR TIRADS in our study have 100% chance of having benign results (NPV) which is higher than other studies such as Dy and Kasala et al., (87.5%)[15] Ha et al., (90.1%)[16] Grani et al., (97.8%)[17] and Horvath et al., (88%).[11]

When using the ATA guidelines for predicting thyroid malignancy in our study, it had a sensitivity of 88.2% which is similar with Ha et al., (89.6%)[16] and higher than Grani et al., (75%)[17] specificity of 57.8% which is noted to be higher than Ha et al., (33.2%)[16] and Grani et al., (45.3%).[17] The overall accuracy of ATA guidelines in predicting thyroid malignancy in our study is 60.4% which is higher than Ha et al., (46%).[16] Using ATA, the probability that FNA recommended nodules are malignant in the FNAC is 16.5% (PPV) which is higher with Grani et al., (9.6%)[17] but lower than Ha et al., (28.3%).[16] Nodules that are not recommended for FNA have 98.1% chance of having benign results (NPV) which is higher than Ha et al., (91.6%)[16] and Grani et al., (95.9%).[17]

In summary, the ACR TIRADS classification when compared to the ATA guidelines had high sensitivity (100% vs 88.2%) in which the latter had more false negative results. The ACR TIRADS classification was less specific (52.2%% vs 57.8%) when compared to ATA in which the former had more false positive results. Both had equal PPV (10.9% vs 10.5%) and NPV (100% vs 98.1%) was slightly lower for the ATA. The ACR TIRADS had inferior overall accuracy (56.4% vs 60.4%) as compared to ATA in which the latter had more correctly identified nodules.

CONCLUSION

In conclusion, the ACR TIRADS and ATA guidelines provided the usefulness of ultrasound based risk stratifications of thyroid malignancy. The diagnostic performances of ultrasound-based risk stratification tools differed between ACR TIRADS and ATA guidelines. The ACR TIRADS classifications appears to be more sensitive than the ATA classification. The ATA guidelines on the other hand proves to be more specific test. Each tool has its unique advantages and disadvantages. Therefore, clinicians must use these tools with utmost vigilance to avoid over or under diagnosis and to avoid unnecessary thyroid nodule biopsies.

Limitations, Strengths and Recommendations

This study had several identified limitations. First, the gold standard used in this study is FNA cytology and can yield a false-negative result of up to 3.7% based on meta-analysis,[18] however, it would be unethical to surgically resect all nodules included in this study and confirm the diagnosis. Second, there might be an overestimation of the proportion of nodules with malignancy since this is based on Bethesda Class IV to VI, rather than Bethesda VI alone or the surgical pathology report since not all patients underwent surgery. Third, we had a small sample size as compared to other bigger studies because it was underestimated in the initial sample size calculation. Fourth, this study was done in a single institution which may reflect the relatively small sample size and might not be representative of the entire population. Fifth, the nodules for biopsy were already flagged by the referring physician, the criteria for classification of these nodules were not known.

The major strength of this study is that the nodules that were for biopsy were examined in real-time ultrasonography before sample is obtained and as compared to retrospective studies, we are confident that the nodules being biopsied are the nodules being sonographically classified.

As for our recommendations, a prospective multicenter study and a longer duration of study is highly recommended to achieve a greater number of subjects.

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Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

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APPENDICES

Appendix A. American College of Radiology – Thyroid Imaging, Reporting and Data System (ACR- TIRADS)[14]

Composition		Echogenicity		Shape		Margin		Echogenic Foci	
Cystic or almost completely cystic	0	Anechoic	0	Wider-than-tall	0	Smooth	0	None or large comet tail artifacts	0
Spongiform	0	Hyperechoic or isoechoic	1	Taller-than-wide	3	Ill-defined	0	Macrocalcifications	1
Mixed Cystic or Solid	1	Hypoechoic	2			Lobulated or irregular	2	Peripheral (rim) calcifications	2
Solid or almost completely solid	2	Very Hypoechoic	3			Extra-thyroidal extension	3	Punctate echogenic foci	3

Points	0 points	2 points	3 points	4 to 6 points	7 points or more
TIRADS Scores	TIRADS 1	TIRADS 2	TIRADS 3	TIRADS 4	TIRADS 5
Interpretation	Benign	Not Suspicious	Mildly Suspicious	Moderately Suspicious	Highly Suspicious
Recommendation	No FNA	No FNA	FNA if ≥ 2.5 cm Follow up if ≥ 1.5 cm	FNA if ≥ 1.5 cm Follow up if ≥ 1cm	FNA if ≥ 1 cm Follow up if ≥ 0.5 cm

Appendix B. Sonographic patterns, estimated risk of malignancy, and fine-needle aspiration guidance for thyroid nodules based on American Thyroid Association management guidelines for adult patients with thyroid nodules[4]

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

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CASE REPORT

Rare Presentation of Right Adrenal Mass: Extramedullary Haematopoiesis in a Patient with Thalassaemia Intermedia

Poh Shean Wong,[1] Lit Sin Yong,[1] Nor Afidah Binti Karim,[1] Ee Leng Gan,[2] See Guan Toh,[2] Noor Lita Binti Adam[1]

[1]*Endocrinology Unit, Department of Medicine, Hospital Tuanku Ja'afar Seremban, Malaysia*
[2]*Haematology Unit, Department of Medicine, Hospital Tuanku Ja'afar Seremban, Malaysia*

Abstract

Extramedullary hematopoiesis (EMH) is a rare cause of adrenal mass. We present a 44-year-old woman who has thalassaemia intermedia, referred to Endocrinology clinic for huge adrenal mass. Along with a paraspinal lesion discovered in this patient, the leading diagnosis was EMH. The patient was treated with hypertransfusion and hydroxyurea, which led to a reduction in the size of the right adrenal mass and paraspinal mass.

This case highlights the challenges in managing this rare condition. Although EMH is a rare cause of adrenal mass, the diagnosis must be considered in any patient with a history of a congenital hemolytic disorder, to avoid unnecessary surgical procedures.

Key words: adrenal mass, adrenal incidentaloma, extramedullary hematopoiesis, thalassaemia, congenital haemolytic disorder

INTRODUCTION

Thalassaemia is one of the most common autosomal recessive disorders and is highly prevalent in countries within the tropical belt, including Malaysia.[1,2] In Malaysia, thalassaemia is the most common inherited blood disorder.[3]

Extramedullary hematopoiesis (EMH) is a well-documented manifestation of thalassemia, as well as other severe disorders of hematopoiesis.[4] The usual sites involved include the liver, spleen and paraspinal regions. The adrenal as a site of extramedullary haematopoiesis is rarely seen.[5]

Herein, we report a rare case of huge right adrenal mass in a 44-year-old woman who has inherited hemoglobinopathy.

CASE

A 44-year-old female with underlying essential hypertension since 2016, was diagnosed to have thalassaemia intermedia since 2002 at the age of 27 years old. She required infrequent blood transfusion until 2017.

Her DNA analysis of alpha and beta globin genes detected the presence of a single alpha gene deletion, together with compound heterozygous state for β^{+}-thalassaemia and Siriraj $^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-thalassaemia. This patient has a vague abdominal mass since 2016. The ultrasound of hepatobiliary system showed a right liver lobe mass.

A computed tomography of the abdomen was performed which revealed incidental finding of a right adrenal mass. The patient was then referred to the Endocrinology clinic for further workup of the adrenal mass.

Otherwise, the patient did not have symptoms of anaemia. She did not experience excessive weight gain, easy bruising or fracture. She denied headache, palpitation, flushing, abdominal pain, tremor or anxiety. Her menses were regular with no history of menorrhagia or dysmenorrhea. She had no history of surgery. Her younger brother was diagnosed to have thalassaemia intermedia and was on regular blood transfusion. She is a housewife. She neither smokes nor drinks alcohol.

On physical examination, she had mild pallor and jaundice but no cushingoid feature. Her weight was 56.1 kg, height was 158 cm, with BMI of 22.5 kg/m^2. Abdominal examination revealed fullness of the abdominal right upper quadrant with large palpable firm mass, and huge splenomegaly extending inferomedially to umbilical level. Cardiovascular and respiratory examinations were unremarkable. There was no neurological deficit.

Blood investigation showed chronic microcytic hypochromic anemia with hemoglobin level of 7.6–9.9 g/dL on different occasions. Other blood parameters are shown in Table 1. Her adrenal hormone assessment was normal (Table 2).

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Corresponding author: Dr. Poh Shean Wong, MRCP
Endocrine Fellow
Hospital Tuanku Ja'afar Seremban
Jalan Rasah, Bukit Rasah, 70300 Seremban, Negeri Sembilan, Malaysia
Tel. No.: (+60) 06-7684000
Fax No.: (+60) 06-7685072
E-mail address: pswongboey@gmail.com
ORCiD: https://orcid.org/0000-0002-4686-5515

Table 1. Initial blood investigations

	Value	Reference value
Total bilirubin	54	5-21 µmol/L
Alanine transaminase	21	10-49 U/L
Alkaline phosphatase	52	46-116 U/L
Albumin	44	32-48 g/L
Sodium	141	136-145 mmol /L
Potassium	3.5	3.5 to 5.1 mmol /L
Creatinine	45 µmol/L	
Serum ferritin	1313.65	10-291 µg/L
Tumor marker	CA125: 5.1 CA19-9: 7.7 Alpha fetoprotein: < 1.1 CEA: 3.3	CA 125: <35 U/mL CA19-9: <37 U/mL Alpha fetoprotein: < 6.7 IU/mL CEA: <5 ug/L
TSH	2.96	0.55 to 4.78 mIU /L
Free T4	15.7	11.5-22.7 pmol/L

Table 2. Adrenal hormonal investigations

	Value	Reference value
Cortisol	212.7	119-618 nmol/L
ACTH level	3.7	<10.2 pmol/L
Serum Dehydroepian-drosterone sulphate	<0.41	0.95-11.70 µmol/L
Testosterone level	<0.1	0.30-1.70 nmol/L
24 hour - urine catecholamine	Dopamine: 510ug/day (mild elevation of dopamine, not diagnostic of pheochromocytoma) Epinephrine: not detected Norepinephrine: 58.8 ug/day	Dopamine: 64-400 ug/day
Overnight dexamethasone suppression test	16.8 (cortisol suppressed)	<50 nmol/L
Renin Aldosterone	Renin: 11.1 Aldosterone: <103 (Not suggestive of primary aldosteronism)	Renin (mU/L): Supine: 4.2 -59.7 Upright: 5.3-99.1 Aldosterone (pmol/L): Supine: 102.5-858.7 Upright: 102.5-1196.6

Computed tomography (CT) of the thorax, abdomen, pelvis, including adrenal protocol done in year 2018, showed a large heterogenous enhancing mass with necrotic area arising from the right adrenal gland, measuring 11.0x9.8x14.8 cm (Figure 1), with average attenuation of 40 HU from non-contrast CT, absolute and relative washout were 24% and 12% respectively. The adrenal mass was indenting on the segment VI of the liver and right kidney, with the right kidney displaced inferiorly. The left adrenal gland was normal. The radiologist concluded that the right adrenal mass was indeterminate and in view of the patient's history of thalassaemia, the adrenal lesion likely represented adrenal extramedullary hematopoiesis. Besides this, the paraspinal mass also noted from the CT scan was in keeping with extramedullary hematopoiesis (Figures 2 and 3).

A multidisciplinary discussion was held between endocrinologists and haematologists and they concluded that the patient was not suitable for operation owing to high bleeding risk. Subsequently, she was given hypertransfusion, with the aim of achieving a level more than 12 g/dL. Furthermore, this patient was started on iron chelating agent due to iron overload. Hydroxyurea was

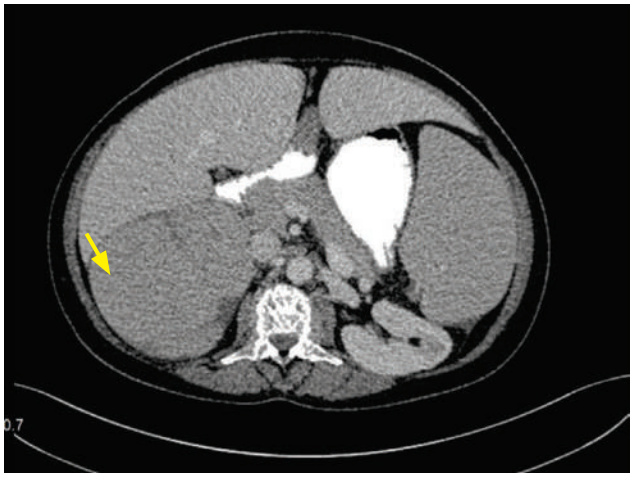

Figure 1. CT scan of the abdomen in 2018 showing large heterogenous enhancing right adrenal mass 11.0x9.8x14.8 cm (yellow arrow).

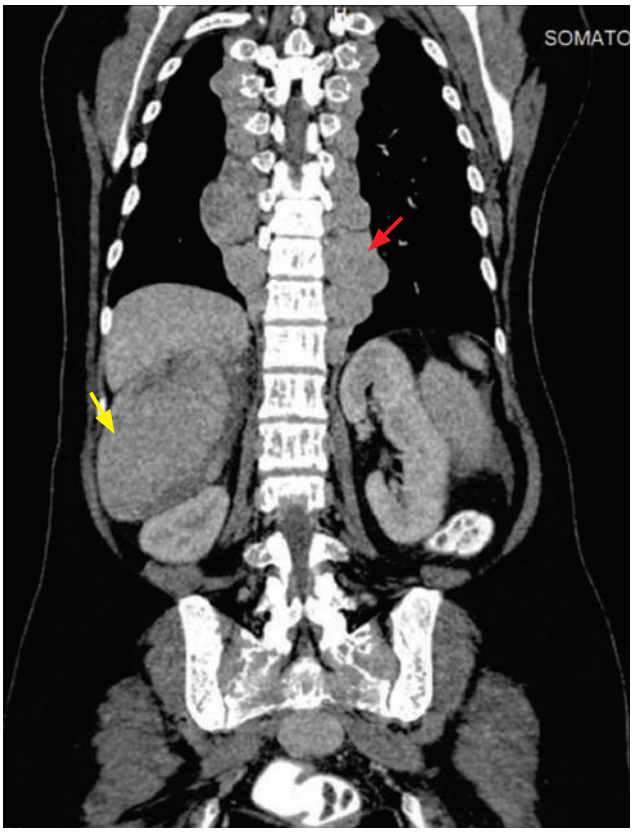

Figure 2. CT scan of thorax, abdomen, pelvis in 2018 revealing paraspinal mass (red arrow). Right kidney is displaced inferiorly by the huge right adrenal mass (yellow arrow).

commenced as part of the treatment for extramedullary haematopoiesis. Computed tomography scan of the abdomen was repeated in year 2020 and showed size reduction of the right adrenal mass and paraspinal mass (Figure 4).

DISCUSSION

The proportion of adrenal mass discovered incidentally on imaging studies was estimated to be 1–5% of all

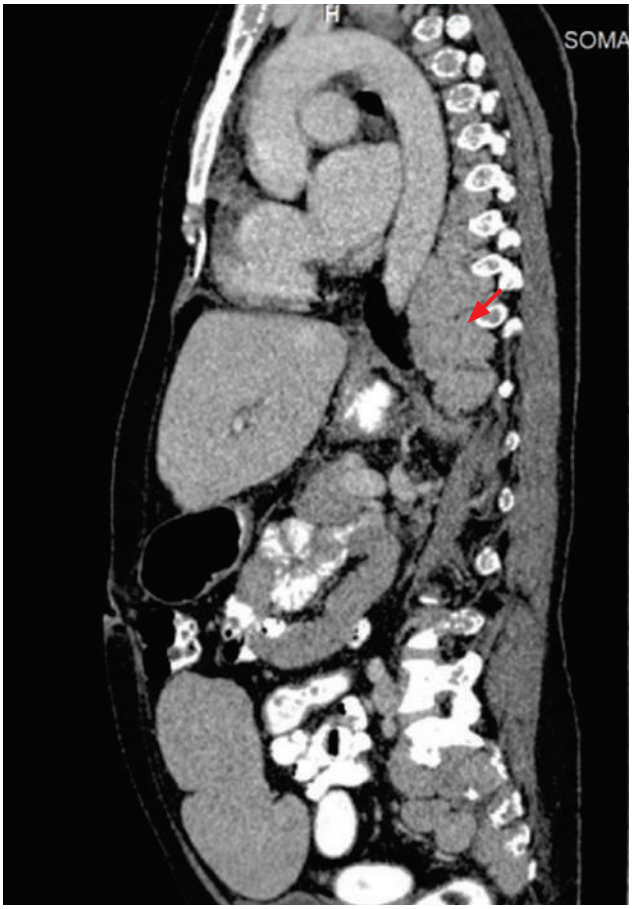

Figure 3. CT scan of thorax, abdomen, pelvis in 2018 (sagittal view) showing paraspinal mass (red arrow).

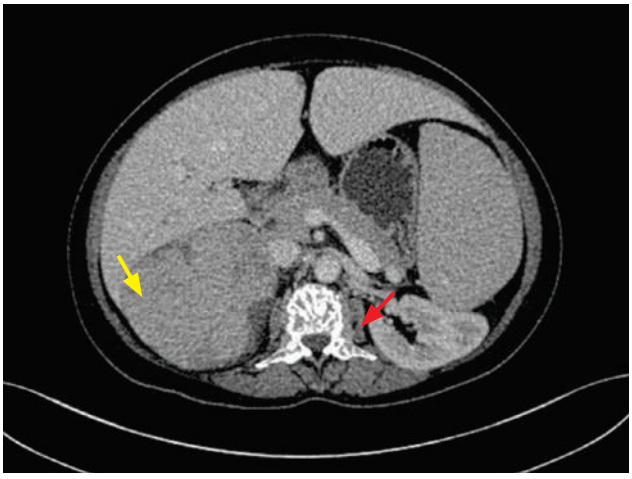

Figure 4. CT scan of the abdomen in 2020 showing size reduction of the right adrenal mass 9.8 x 8.8 x 13.2 cm (yellow arrow) and paraspinal mass (red arrow).

abdominal CT scans performed.[6] In our case, the patient was referred to endocrinology team for an adrenal incidentaloma. Given the imaging findings of the incidental adrenal lesion and paraspinal lesion discovered in a patient with thalassaemia, the leading diagnosis is extramedullary hematopoiesis.

This patient did not have underlying primary malignancy to suggest metastatic disease as one of the differential diagnoses. Adrenal adenoma is unlikely in this case, nor is it associated with paraspinal lesion. The adrenal lesion is huge with its attenuation of 40 HU from the non-contrast CT. Although the adrenal lesion in this patient is huge, other CT features are not suggestive of adrenocortical carcinoma, where irregular shaped, stellar central hypodensity and capsular enhancement are expected.

Extramedullary hematopoiesis (EMH) is a physiological compensatory phenomenon in response to altered hematopoiesis occurring secondary to inadequate bone marrow function. EMH in the medical literature was commonly found in the liver and spleen whereas it is rarely seen in adrenal glands, breast, dura mater, and bowel.[5,7-9]

It often occurs in hemoglobinopathies, hemolytic anemias and myeloproliferative disorders.[10]

Pathological causes of EMH in the adrenal gland were described in multiple case reports, which included the defects in hemoglobin production associated with sickle-cell disease,[9] hemoglobin H constant spring disease,[11] thalassemia[8,12-16] and impaired red blood cell membrane production linked with hereditary spherocytosis.[17]

The exact mechanism of EMH in the adrenal gland is unknown, but several hypotheses are suggested. The adrenal gland has hematopoietic capacity during the fetal period and EMH may develop from primitive rests in disease conditions. Other scientists believe that embolization of hematopoietic stem cells and homing in adrenal gland may occur. Chronic hypoxia is another presumptive cause of EMH.[12,18,19]

Adrenal EMH might be clinically detected as incidentaloma, as happened in our case.[12] Adrenal incidentaloma in association with hematologic disorders, e.g., agnogenic myeloid aplasia or beta thalassemia, needs careful imaging as well as adrenal hormonal investigations, in order to exclude malignancy and subclinical hypersecretory syndromes. Although EMH is a rare cause of an adrenal mass, the diagnosis must be considered in any patient with a history of a congenital haemolytic disorder, to avoid unnecessary surgical procedures.[13]

CT adrenal of this patient showed a large heterogenous enhancing mass with necrotic area arising from the right adrenal gland. There are no specific diagnostic findings of extramedullary haematopoiesis in imaging studies.[20] Adrenal EMH may appear as a homogeneous mass in ultrasonography (USG)/CT or as a heterogeneous mass with cystic change and calcification.[8,11]

Although biopsy remains the gold standard for establishing a tissue diagnosis, it is an invasive procedure that carries the risk of catastrophic haemorrhage and is therefore not usually advocated.[21-23] The Clinical Practice Guideline of the European Society of Endocrinology recommended against the use of an adrenal biopsy in the diagnostic work-up of patients with adrenal masses unless there is history of extra-adrenal malignancy.[24] In this case, along with the paraspinal lesion, the leading diagnosis is extramedullary hematopoiesis. Hence, the patient's clinical details and radiological pictures are extremely crucial in diagnosis.

In our case, EMH involved the right adrenal gland. It is interesting that our literature review on EMH also reported right-side predilection, where 13 out of 17 cases of EMH involved the right adrenal gland,[8,11-15,17,25-30] two cases involved the left adrenal gland[6,9] and two cases involved bilateral adrenal glands.[16,31]

A rather striking right-sided predominance was noted in a study done by Kenney et al., where 53 of 72 adrenal myelolipomas involved the right adrenal glands.[32] Besides, in an analysis of adrenal myelolipoma conducted by Decmann et al., 260 tumors (59.2%) were on the right side, 111 on the left side (25.3%), while 54 tumors (12.3%) were bilateral.[33]

Although speculative, a possible explanation is that asymptomatic right-sided adrenal masses are more likely to be detected incidentally, particularly at ultrasound, owing to the fact that the right adrenal region is seen clearly during ultrasound examination of the gallbladder. It is not always possible to visualize the normal adrenal glands (especially on the left side) with an ultrasound.[34,35]

Treatment options for patients with EMH are described for thalassemia patients and depend on the location and symptoms. Different approaches included surgery, local radiation, blood transfusion and hydroxyurea.

There are published reports of incidentally detected adrenal masses in patients with haematological disorders, where adrenalectomy was performed, resulting in the histological surprise of EMH.[8,11,12,17,25] Surgical excision is recommended in patients who are symptomatic and resistant to other modalities.[15] However, the disadvantages of the surgical intervention include risk of excessive bleeding due to the high vascularity of the mass and high incidence of recurrence.[36] In this case, patient is asymptomatic and hence, she was treated medically.

The medical approach involves hypertransfusion and oral hydroxyurea. Blood transfusion corrects the anaemia, and therefore the need for extramedullary haematopoiesis decreases, resulting in the relative inactivity of these tissues and their shrinkage. This is probably due to a decrease in blood flow in these tissues, rather than their actual atrophy.[37]

Hydroxyurea is a ribonucleotide reductase enzyme inhibitor. By reducing the globin chain imbalance through stimulating synthesis of fetal hemoglobin and cytoreduction, hydroxyurea contributes to a decrease in ineffective erythropoiesis and the associated EMH.[38,39] To date, there is no reported data on radiotherapy of the adrenal gland.

For paraspinal/epidural lesions, asymptomatic disease may require no specific treatment, whereas relative low dose radiation therapy is suggested in symptomatic cases because the hematopoietic tissue is notably radiosensitive and can lead to marked shrinkage of the mass and rapid neurologic improvement.[40] However, Aliberti et al., reported two thalassaemic patients with spinal cord compression due to extramedullary haematopoiesis who achieved complete regression with blood hypertransfusion therapy.[41] In the case presented above, the patient was given hypertransfusion and hydroxyurea. There was subsequent reduction in size of the right adrenal mass and paraspinal mass.

CONCLUSION

In conclusion, we presented a patient with thalassaemia intermedia who was referred due to an adrenal incidentaloma. With the background history of thalassaemia along with the presence of a paraspinal lesion, the leading diagnosis is extramedullary hematopoiesis. Although EMH is a rare cause of adrenal mass, the diagnosis must be considered in any patient with a history of a congenital hemolytic disorder, to avoid unnecessary surgical procedures.

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

Statement of Authorship
All certified fulfilment of ICMJE authorship criteria.

Author Disclosure
The authors declared no conflict of interest.

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CASE REPORT

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Severe Hypothyroxinemia in a Young Adult with Carbimazole-Treated T3-Predominant Graves' Hyperthyroidism, Reversed with L-Thyroxine Loading Immediately Post-Total Thyroidectomy

Brenda Chiang[1] and Yin Chian Kon[2]

[1]*Department of General Medicine/Endocrinology, Sengkang General Hospital, Singapore*
[2]*Department of Endocrinology, Tan Tock Seng Hospital, Singapore*

Abstract

Patients with triiodothyronine (T3)-predominant Graves' hyperthyroidism with markedly elevated serum thyroid stimulating immunoglobulin (TSI) levels and massive goitre may display discordant hypothyroxinemia with eutriiodothyroninemia or hypertriiodothyroninemia while on anti-thyroid drug therapy. A 25-year-old female with the above was started on oral carbimazole therapy for 9 months before total thyroidectomy. Preoperatively, her serum free T4 was reduced to below detection limit, and total T4 reduced to 11% of lower limit of normal, while T3 levels remained normal, and TSH remained largely suppressed. Immediately after total-thyroidectomy, a loading dose of L-thyroxine (L-T4) was administered intravenously. She was extubated without any postoperative complications. Serum free and total T4, and TSH normalized within the next 24 hours. The peculiar thyroid axis dynamics and use of L-T4 postoperative loading in such a rare clinical scenario are discussed.

Key words: Graves' disease, hypothyroxinemia, T3-thyrotoxicosis, L-thyroxin

INTRODUCTION

Triiodothyronine (T3)-predominant Graves' disease was first described in 1984 and is defined by persistently elevated serum free T3 levels associated with hypothyroxinemia in Graves' disease patients who are on anti-thyroid-drug (ATD) therapy.[1] It occurs in about 5% of Graves' thyrotoxic patients[2] and is most often found in patients with higher thyroid stimulating immunoglobulin (TSI) levels together with larger thyroid glands that are iodine avid, have higher TPO activity and a lower thyroglobulin-iodine content after treatment.[3]

CASE

Our patient is a 25-year-old, married female, who was diagnosed with Graves' disease at age 18 years. She had completed a 3-year course of carbimazole (CMZ) abroad, but suffered a relapse 6 months later, whereupon CMZ was restarted by her doctor. Five months before presenting to us, her thyroid function was normal on CMZ 5 mg every other day. She presented with 8 kg weight loss, palpitations and vomiting over the last 2 months, whilst compliant on low dose CMZ. She was not trying to conceive. On examination, she was alert with a GCS score of 15. Her blood pressure was 131/79 mmHg, heart rate 135 beats/min, regular. She weighed 40kg and her body mass index was 17.5.m^2. Her skin felt warm and sweaty, and she had fine tremors on outstretched hands. She had a WHO Grade 3 goitre, but signs of thyroid eye disease were absent. She denied any symptoms of dysphagia, difficulty breathing nor change in her voice. Her serum fT4 measured greater than 154.8 pmol/L (RR 8-21), fT3 greater than 30.8 pmol/L (RR 4.8-8.3) and thyroid stimulating hormone (TSH) 0.01mIU/L (0.34-5.6). Her thyrotrophin receptor antibody (TRAb) level was greater than 40 IU/L (<1.0). Ultrasound thyroid scan revealed an enlarged thyroid gland with very heterogenous parenchyma and increased vascularity. The right lobe measured 10.9x5.0x5.5 cm, left lobe 9.0x5.0x3.0 cm and isthmus 8.0x3.7x2.6 cm. No thyroid nodules were seen on ultrasound.

Her dose of CMZ was increased to 100 mg daily for a week then reduced to 30 mg daily. The course of her weight, heart rate, thyroid function test (TFT) and CMZ daily dosage are depicted in Figures 1A to C respectively. Over a span of 7 months, her serum TSH showed inappropriate suppressed or inappropriate normal levels when fT4 was low and T3 normal, as well as transient appropriate suppression due to T3 toxicosis (Figure 1B). She was advised to take appropriate contraceptive measures in view of her unstable TFTs, and scheduled for total thyroidectomy as definitive therapy for T3-predominant Graves' disease. She was well and clinically euthyroid on elective admission. Laboratory tests performed pre-operatively showed serum free T4 less than 2 pmol/L (8-21), total T4 10 nmo/L (70-140), free T3 4.0 pmol/L (4.8-8.3), total T3 2.07 nmol/L (0.9-2.6), TSH 16.9 mIU/L (0.34-5.6), TRAb >40 IU/L (<1.0), and TSI >5000% (<179).

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Corresponding author: Yin Chian Kon, MBBS, BSc, FAMS, FRCP (Edin), FACE (USA)
Senior Consultant, Department of Endocrinology, Tan Tock Seng Hospital
11 Jalan Tan Tock Seng, Singapore 308433
Tel. No.: 65-81263173
Fax No.: 65-63573087
E-mail: Winston_Kon@ttsh.com.sg
ORCiD: https://oricd.org/0000-0002-9693-2587

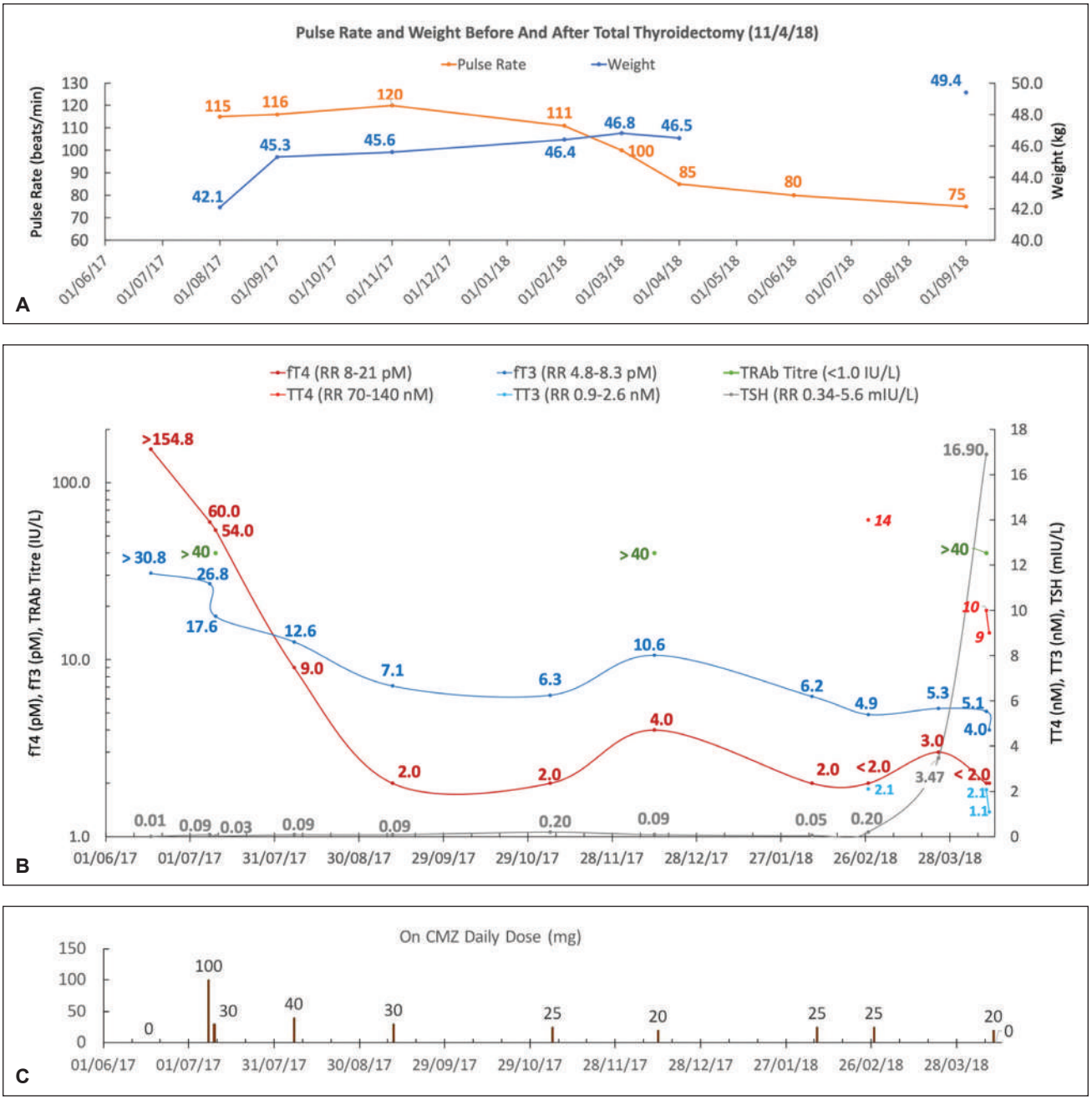

Figure 1. Serial **(A)** weight and heart rate; **(B)** thyroid function and TRAb titres and **(C)** daily dose of oral carbimazole (CMZ) therapy, in a patient with T3-predominant Graves' hyperthyroidism. TSH was suppressed from 11/9/17 to 6/11/17 (8 weeks with low fT4 and normal fT3), further suppressed on 13/12/17 (T3 toxicosis) and went from suppressed to normal from 7/2/18 to 24/3/18 (6 weeks with low fT4 and normal T3).

In the operating theatre, immediately after her thyroid was excised, in view of her severely depleted total T4, intravenous L-thyroxine (L-T4) 500 mcg (10 mcg/kg) was administered as a loading dose to quickly restore her circulating T4 reservoir. Her perioperative thyroid function tests in the first 24-hours post total-thyroidectomy are depicted in Figure 2. Post-operatively, there was no decreased ventilatory drive and she was extubated successfully. Other hypothyroid signs and symptoms were also absent. She was monitored closely for adverse effects such as cardiac ischaemia and infarction, tachycardia and palpitations. No immediate post-operative complications were observed. Her resected thyroid gland weighed 231 grams.

She was discharged well on the 3rd postoperative day. She remained euthyroid (fT4 12-16 pmol/L, TSH 0.53-4.34 mIU/L) on oral thyroxine replacement and conceived 21 months later. Her thyroxine dosage was optimized throughout pregnancy. Her TRAb levels had reduced to 3.2 IU/L at 34 weeks' gestation and she gave birth to a term healthy baby boy.

DISCUSSION

Normally, T4 and T3 are secreted by the thyroid in a ratio of 11:1, with the rest of circulating T3 produced extrathyroidally.[4] In Graves' hyperthyroidism, there is an increase in the intrathyroidal deiodination of T4 to

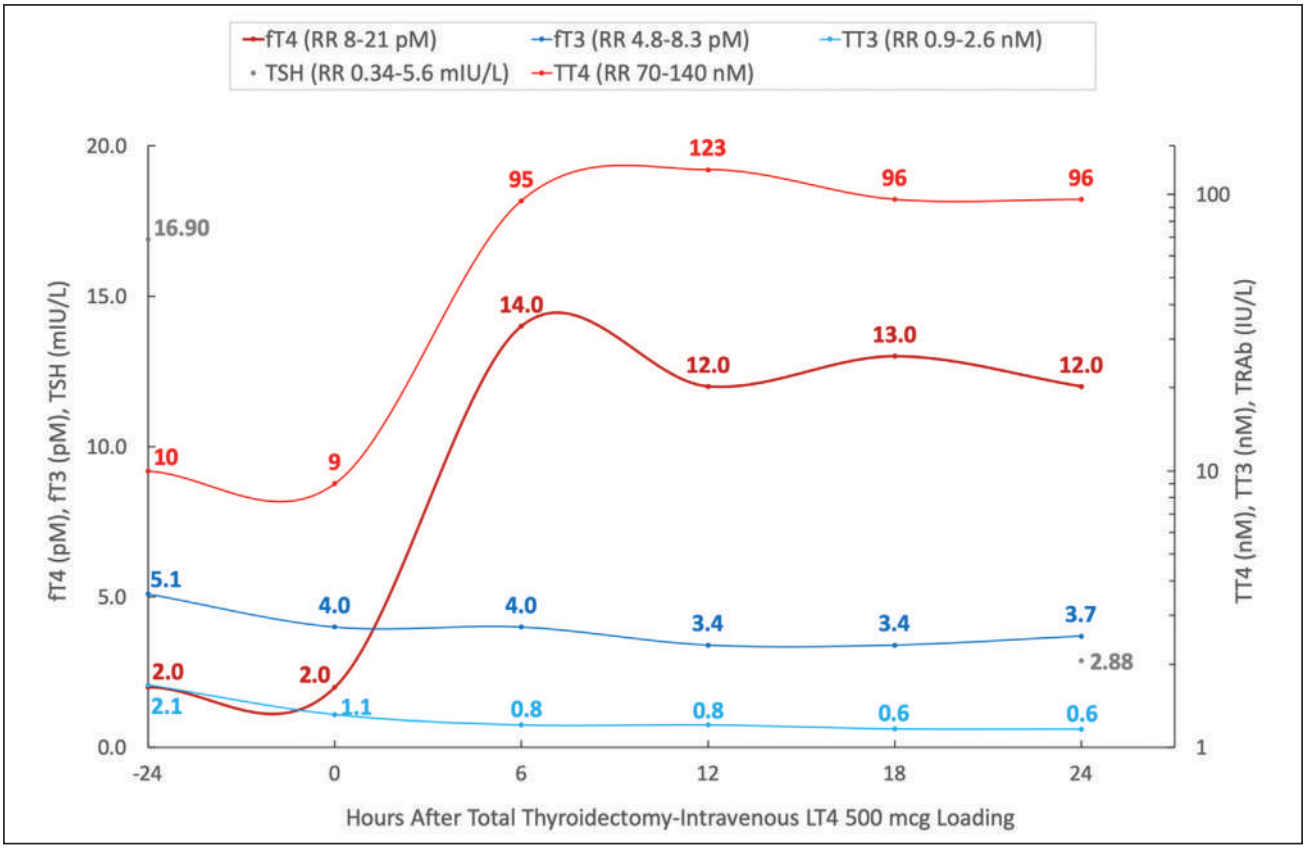

Figure 2. Thyroid function profile before and within 24-hours after total thyroidectomy with intravenous levothyroxine 500 mcg loading.

T3.[5] The higher levels of TSI in T3-predominant Graves' disease result in greater stimulation and higher thyroid peroxidase (TPO) activity levels, higher iodine turnover and lower intrathyroidal iodine pool, and thus preferential T3 production.[6]

When serum fT4 is low and serum fT3 normal, hypothalamic-tancyte and pituitary-thyrotroph intracellular T3 levels should decrease, due to decreased availability of T4 for local conversion to T3 by type 2 iodothyronine deiodinase (DIO2).[7-9] This should lead to an increase in serum TSH by negative feedback. In this case, serum TSH remained suppressed at times over a period of 24 weeks (Figure 1B). This is attributed to mild T3 toxicosis with delayed TSH recovery. However, it is speculated that besides driving high thyroidal iodine turnover, a high TRAb level may downregulate pituitary TSH secretion by an ultra-short negative feedback loop by acting on the pituitary TSH receptor (TSHR).[10,11] The pituitary TSHR, expressed in the human anterior pituitary on folliculo-stellate cells, lies outside the blood-brain barrier and is therefore accessible to these autoantibodies.[12] As the pituitary TSHR is also recognized by TSI, this interaction plausibly explains the prolonged serum TSH suppression despite serum fT4 being low and T3 being normal, until central regulatory tissue T3 levels were low enough to override the suppressive effect of TSI (Figure 1B). Chung et al.,'s retrospective study of Graves' patients on long-term ATD has provided some indirect evidence to support this hypothesis. They showed that after both T4 and T3 had been normal for 3, 6 and 12 months whilst on ATD therapy, serum TSH was inversely correlated with TRAb activities (r= -0.353, p<0.001 at 3 months; -0.317, 6 months; and -0.297, 12 months).[13]

High levels of TSI on concomitant ATD treatment leads to hypothyroxinemia with increased circulating T3 to T4 ratios due to intrathyroidal iodine depletion.[1-3] The intriguing dynamics poses a therapeutic challenge to the clinician: should one aim to normalize TSH that would entail hypothyroxinemia, or target normal T4 that would entail T3 toxicosis (Figure 1B). In a seminal study by Pedraza et al., rats were rendered hypothyroxinemic to varying degrees by being fed different grades of iodine-deficient diets. When plasma total T4 was decreased to 25% of normal, plasma TSH had increased 6-fold, whereas plasma total T3 remained normal. When plasma total T4 was decreased to 5% of normal, plasma TSH had risen 10-fold, and plasma total T3 reduced to 46% of normal. Intriguingly, tissue T3 levels were demonstrated to be tissue specific, being normal in muscle and heart, elevated in lung and ovary, but low in brain, pituitary and brown fat, in the same animal.[14] Notably, tissues depending on plasma T3 would have normal T3 concentrations, while tissues that depend more on circulating T4 for the local generation of T3 would be less protected from hypothyroidism.[9,14] Moreover, Fonseca et al., have elegantly shown in a DIO2-knockout mouse model that the hypothalamic-thyroid axis is wired to maintain normal plasma T3 levels, through coordination of T4-to-T3 conversion between thyrotrophs and hypothalamus.[15] Accordingly, we suggest that in the present clinical context, the priority target for ATD dose titration should be circulating fT3 level at upper normal.

During early pregnancy, fetal brain development depends more on circulating maternal T4 than T3.[16] When maternal serum T4 levels are low and T3 levels are normal or high, as seen in this case or when a patient is taking T3 supplements, fetal brain development might be adversely affected. Conversely, treating to high normal maternal fT4 levels to avoid ATD-induced fetal hypothyroidism would entail prolonged maternal T3 toxicosis. To avoid being faced with such a therapeutic dilemma, we suggest that reproductive-age female patients who present with large goitre and very high TSI levels leading to T3-predominant Graves' hyperthyroidism, should undergo early definitive therapy by total thyroidectomy followed by L-thyroxine replacement, before trying to conceive. They should also be advised to take appropriate contraceptive measures before definitive surgical therapy.

Greater than 99.9% of circulating T4 is protein-bound. When preoperative total T4 was very low at 9 nM (70-140), starting our patient on daily maintenance doses of 75-100 mcg L-T4 after total thyroidectomy would have meant a slow correction of fT4, as binding sites on thyroxine-binding globulin, transthyretin and albumin are being saturated. Extrapolating from early clinical studies of patients with myxoedema coma where the mean thyroxine deficit was estimated at 360 mcg, and assuming an euthyroid daily T4 turnover of 80 mcg, a daily maintenance dose of 100 mcg would take about 18 days to replete the extrathyroidal T4 pool.[17] A study by Arlot et al., showed that patients with myxoedema coma who were loaded with oral L-T4 500 mcg followed by 100 ug daily, did not achieve normal total T3 nor normal total T4 blood levels within 14 days.[18] In contrast, Ridgway et al., showed that in 7 non-comatose hypothyroid patients with mean total T4 11.6 nM (51-141), given IV L-T4 at mean loading dose of 428 mcg and then 100 mcg daily, total T4 levels were normalized within 24 hours, as in our case.[19]

Unlike the case studies mentioned above, our patient was clinically euthyroid preoperatively, with normal T3 levels. However, her TSH had risen over 16 days from 3.37 mIU/L to 17.0 mIU/L at 24-hours before surgery, i.e., classic primary hypothyroid pattern. We were concerned that starting with the usual daily maintenance L-T4 dose and the consequent slow correction of T4 would adversely affect her postoperative recovery. Thyroid cancer patients who were rendered short-term hypothyroid by withdrawing both L-T4 and L-T3 therapy for 2-3 weeks were shown to have normal baseline minute ventilation, but depressed hypoxic[20] and hypercarbic[21] ventilatory drives that normalized after resuming thyroid hormone replacement therapy. There was also a potential risk of depressed cardiac function and increased systemic vascular resistance post-thyroidectomy.[22] Hence, we opted to administer an intravenous loading dose of L-T4, with no risk of aspiration, to quickly replete extrathyroidal T4 pool, and thereby allow maintenance of adequate T3 levels by peripheral conversion as calorie intake resumed to normal postoperatively (Figure 2).

As our patient was clinically euthyroid preoperatively, with normal T3 levels, another option would have been to give a loading dose of oral L-T4 after full recovery from anaesthesia. It has been shown that within 2 to 3 hours of oral ingestion of either a weight-based[23] or 1000 mcg[24] of L-T4, significant rises of fT4 to adequate levels may be attained. This is seen in a test setting to rule out malabsorption in patients with medication nonadherence who remain biochemically hypothyroid despite "taking" high doses of L-T4. Moreover, Rajendran et al., have described their institutional experience of successfully treating 13 out of 14 patients with myxoedema coma using oral L-T4 loading therapy.[25]

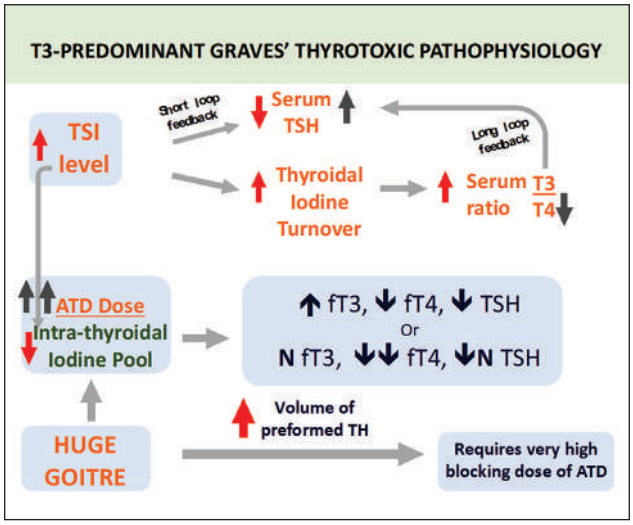

Figure 3. Proposed pathophysiology of T3-predominant Graves' thyrotoxicosis with discordant thyroid function.

Our patient was young with no history of cardiovascular diseases nor arrhythmias. If our patient was elderly or with heart disease, one would be more circumspect about LT4 loading, to avoid precipitation of myocardial ischemia and cardiac failure. Although total thyroidectomy would have removed our patient's thyroidal source of T3, we did not start the patient on liothyronine as she was well preoperatively and we did not expect any postoperative complications nor prolonged reduced calorie intake that might impair peripheral T4 to T3 conversion.

The pathophysiology of T3-predominant Graves' thyrotoxicosis, including how a very high TSI level possibly suppresses TSH in a short feedback loop that leads to discordant thyroid function, is illustrated in Figure 3.

CONCLUSION

This case exemplifies how patients with T3-predominant Graves' hyperthyroidism characterised by markedly elevated serum thyroid stimulating immunoglobulin (TSI) levels and large goitre may display hypertriiodothyroninemia followed by eutriiodothyroninemia with discordant and severe hypothyroxinemia, as ATD therapy is titrated. For the ensuing severe preoperative hypothyroxinemia, we believe this is the first case report demonstrating its safe correction with intravenous levothyroxine loading therapy immediately following total thyroidectomy.

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

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

Author Disclosure
The authors declared no conflict of interest.

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CASE REPORT

Doege-Potter Syndrome: A Presumptive Case of Metastatic Hemangiopericytoma with Persistent Hypoglycemia in a 27-Year-Old Male

Von Lovel Zarra,[1] Kenny Jun Demegillo,[2] May Uyking-Naranjo,[3] Ahmad Domado[1]

[1]*Department of Internal Medicine, Southern Philippines Medical Center, Davao City, Philippines*
[2]*Section of Oncology, Department of Internal Medicine, Southern Philippines Medical Center, Davao City, Philippines*
[3]*Section of Endocrinology, Department of Internal Medicine, Southern Philippines Medical Center, Davao City, Philippines*

Abstract

Doege-Potter syndrome (DPS) is a rare paraneoplastic condition characterized by hypoinsulinemic hypoglycemia from a solitary fibrous tumor. The underlying mechanism is the secretion of a prohormone form of insulin-like growth factor II (IGF-II) by the tumor, which causes decreased release of glucose into the circulation. We report the case of a 27-year-old Filipino male with presumptive DPS from a recurrent right temporo-zygomatic hemangiopericytoma (HPC). The complexity of DPS requires a multidisciplinary approach. Early screening for metastases from HPC may prevent the undesirable sequelae of the disease process.

Key words: Doege-Potter syndrome, hemangiopericytoma, hypoglycemia

INTRODUCTION

Hypoglycemia is a medical emergency commonly caused by medications, alcohol and sepsis. Occasionally, it can be an initial manifestation of a neoplasm, particularly islet cell tumors, such as an insulinoma. In rare instances, it can also be caused by non-islet cell entities such as solitary fibrous tumors (SFT). This condition is called Doege-Potter syndrome (DPS), which accounts for less than 5% of cases.[1]

DPS is a rare paraneoplastic condition characterized by hypoinsulinemic hypoglycemia secondary to ectopic secretion of the prohormone of IGF-II.[2] Only 45 cases of Doege-Potter syndrome have been reported worldwide. Based on an extensive literature search, there are no documented or published cases of DPS from the Philippines.[3]

CASE

A 27-year-old Filipino male presented with a painless, nodular mass on the right temporo-zygomatic area starting 10 years prior to admission. In the ensuing years, he underwent five surgical resection procedures due to recurrences on the same site. Final histopathologic diagnosis revealed hemangiopericytoma (HPC). The patient subsequently underwent radiotherapy and had no recurrence for two years.

He was apparently well until three months before admission when he began to experience malaise. Hospital admission and work-up revealed hypoglycemia, and he was discharged after resolution of the symptom. After a month, he was brought to the emergency room due to generalized tonic-clonic seizure episodes. Further work-up showed hypoglycemia, prompting the consideration of an islet versus non-islet cell tumor etiology. He was discharged and was advised oncologic work-up on an outpatient basis. On the day of admission, he had another seizure episode with deterioration of sensorium.

The patient was not known to have hypertension or diabetes. He had no intake of any glucose-lowering or herbal medications. He did not smoke cigarettes, consume alcohol or use illicit drugs. Family history was unremarkable.

On initial examination, he was drowsy but remained oriented to time, place and person. He was tachycardic (108 beats per minute) and normotensive (110/60 mm Hg), with a respiratory rate of 20 cycles per minute and temperature of 36.1°C. He was also obese, with a body mass index of 30.8 kg/m^2. Pertinent physical findings included a depressed right temporo-zygomatic area and hepatomegaly, with a liver span of approximately 15 cm at the midclavicular line upon palpation. He did not have *caput medusae*, spider angiomata or abdominal tenderness. The other physical and neurologic examination findings were unremarkable. The initial consideration was hypoglycemia secondary to a non-islet cell tumor versus insulinoma. The patient then underwent a series of procedures where informed consent was obtained after risks and benefits were thoroughly explained with appropriate review.

RESULTS

A 72-hour fasting protocol was performed to rule out insulinoma. He experienced neuroglycopenic symptoms on the sixth hour, with a capillary blood glucose (CBG)

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Corresponding author: Von Lovel D. Zarra, MD
Medical Officer III, Department of Internal Medicine
Teresita Perez Hall, 3rd floor ICU Building
Southern Philippines Medical Center
JP Laurel Street, Bajada, Davao City, Davao del Sur 8000
E-mail: von_lobes@yahoo.com
ORCiD: https://orcid.org/0000-0002-4551-9434

of 42 mg/dL. The fasting procedure was then terminated (Figure 1). Insulin and C-peptide levels were decreased, further excluding the possibility of an insulinoma or an endogenous pancreatic source (Table 1). Despite the unavailability of bioassays for IGF-II, the diagnostic algorithm focused on the clinical course of the tumor that caused hypoglycemia. This became helpful in making a presumptive diagnosis.

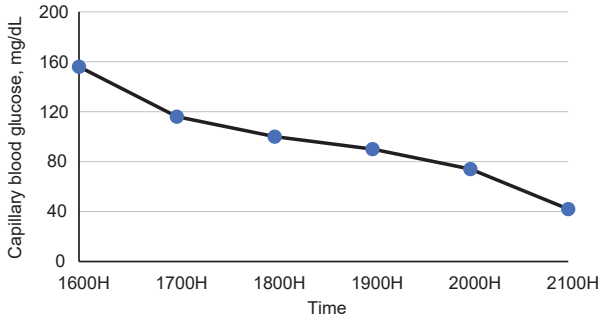

Figure 1. Capillary blood glucose monitoring during the 72-hour fast protocol. Symptomatic hypoglycemia became evident at the sixth hour.

Table 1. Summary of 72-hour fasting protocol

Parameter	Reference range	Pre-fasting	Post-fasting
Glucose, mmol/L	4.10-6.60	6.5 (Normal)	1.93 (Low)
Insulin, pmol/L	2.6-24.9	35.5 (High)	<0.2 (Low)
C-peptide, ng/mL	0.78-5.19	1.19 (Normal)	3.0 (Low)

Cranial computerized tomography (CT) scan showed no evidence of tumor recurrence. However, hepatic and right adrenal metastases without pancreatic involvement were seen on abdominal CT scan. These were described as multiple, well-defined, heterogeneously enhancing ovoid lesions of different sizes predominantly on the right hepatic lobe (Figure 2A). The largest mass was visualized at segment 5 measuring 10.2 cm x 9.1 cm x 10.7 cm. In consideration of the low sensitivity (70 to 90% for all tumor sizes and less than 50% for tumor sizes 1 to 2 cm), variations in sampling, post-operative complications (tumor seeding and bleeding) and limited benefit of liver biopsy, the procedure was not performed in this case.[4]

The right adrenal gland was also found to have a well-defined ovoid lesion measuring 3.7 cm x 4.4 cm x 6.4 cm (Figure 2B). Findings suggestive of metastatic disease of the adrenal gland include a size more than 4 cm (having 80% sensitivity and 60% specificity using a 4 cm cut-off) or a CT density of more than 20 Hounsfield units.[4] The patient's abdominal CT findings were consistent with metastatic disease in conjunction with his clinical progression. Chest CT scan revealed bilateral pulmonary nodules (Figure 3). Skeletal survey, however, was unremarkable.

Slide reviews from prior biopsies of the recurrent temporo-zygomatic mass showed sheets of atypical pericytes with numerous thin-walled, ramifying vessels with some dilations. Connective tissue was very scant (Figure 4). These histologic findings were consistent with hemangiopericytoma. Even without the

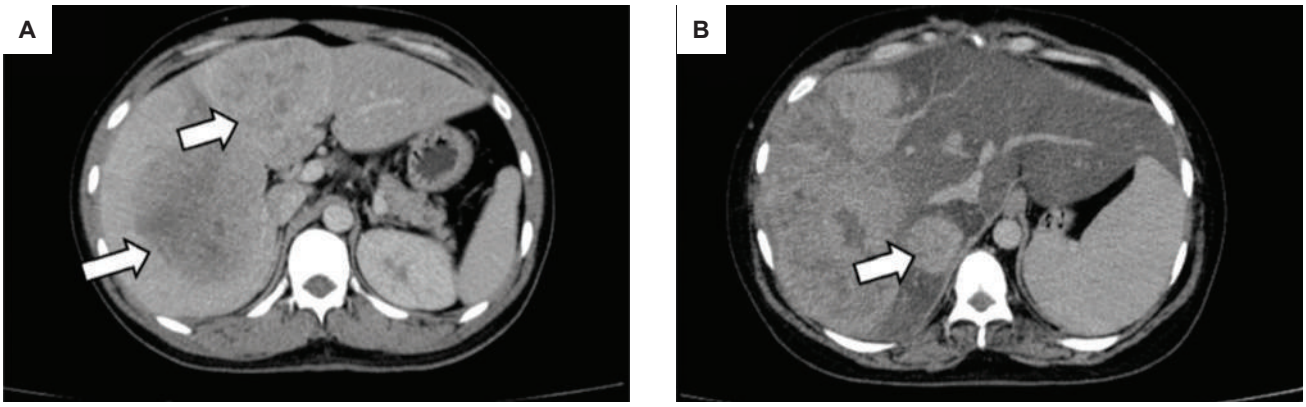

Figure 2. Abdominal computerized tomography scan on axial view showing multiple hepatic **(A)** and right adrenal metastases **(B)**.

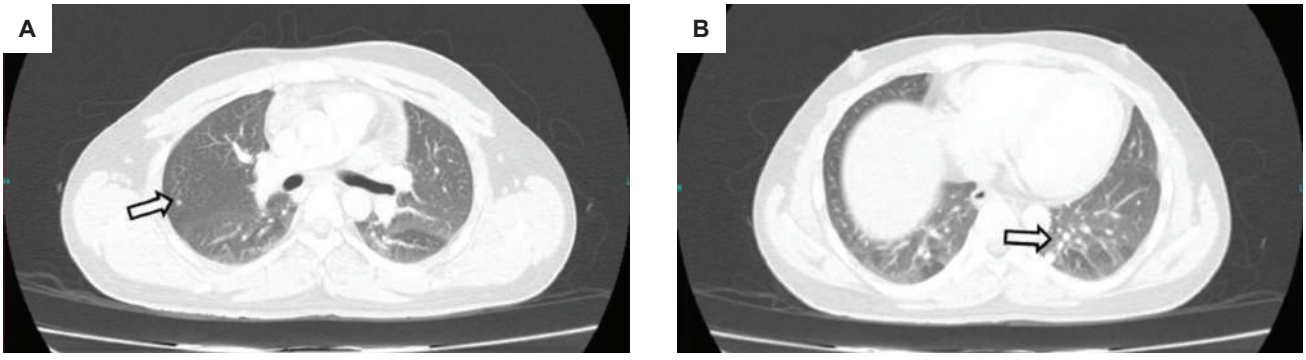

Figure 3. Chest computerized tomography scan in axial view showing bilateral sub-centimeter nodular densities in the apical segment of the right upper lobe **(A)** and postero-basal segment of the left lower lobe **(B)**.

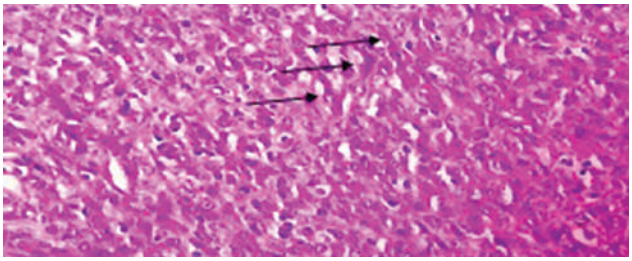

Figure 4. Histopathologic examination of the excised temporo-zygomatic mass. There are pericytes (black arrows) that are round to ovoid, angulated, with hyperchromatic nuclei and basophilic cytoplasm (H&E, 100x).

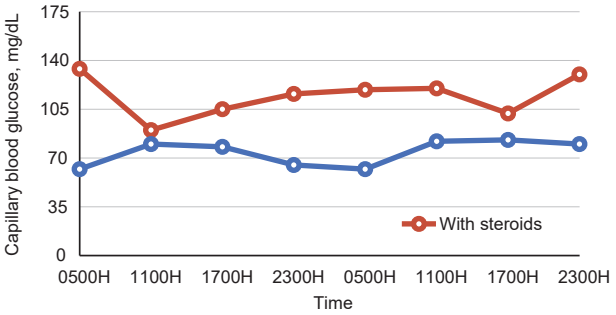

Figure 5. Comparison of 48-hour capillary blood glucose readings with and without Prednisone.

ideal benefit of IGF-II, these findings consolidated the ***presumptive*** diagnosis of hypoglycemia secondary to hemangiopericytoma or solitary fibrous tumor.[1] This is a pattern seen in a rare disease entity called Doege-Potter Syndrome.

To prevent recurrence of hypoglycemia, a continuous infusion of D_5LR 1L with 50cc D_{50}W was given with close monitoring of CBG levels every six hours. Prednisone was also started at 30 mg once a day. After initiation of steroids, there was a significant improvement of CBG levels, with readings ranging from 90 to 125 mg/dL. A comparison of 48-hour CBG results before and after steroid initiation is shown in Figure 5. The patient was also advised an increase in total caloric intake and

The patient was also referred to Surgery service for evaluation. Due to extensive metastases, he was assessed to be a poor candidate for surgery. He was subsequently referred to Oncology service. Weekly doxorubicin was started with no significant response. He was maintained on steroids, initially for one to three months (or indefinitely depending on treatment response) and inpatient glucose infusion for symptomatic states to prevent recurrent hypoglycemic episodes. Weekly outpatient monitoring of his immune system and adrenal imbalance were done after initiation of steroid treatment.

DISCUSSION

This case report presents the complexity and rarity of Doege-Potter Syndrome. Various causes of hypoglycemia, including medications such as insulin, alcohol, sepsis and cortisol deficiency, were considered in the process of arriving at a definite diagnosis.[4] The first three were easily ruled out because the patient had no history of insulin or alcohol use and had no established source of infection. Cortisol deficiency due to right adrenal metastasis was also considered. For adrenal metastases to cause cortisol deficiency, involvement should be bilateral with at least 90% gland destruction.[5] These findings were not present in our patient.

Despite its rarity, endogenous hyperinsulinism became a differential diagnosis. The prototypical cause is an insulinoma, an insulin-secreting pancreatic islet B-cell tumor.[4] This was ruled out after a 72-hour fasting protocol showed decreased post-fasting glucose, insulin and C-peptide levels, in contrast to findings seen in insulinoma (Table 1).[6]

The results of the workup and the patient's temporo-zygomatic mass with liver and right adrenal gland metastases prompted us to consider non-islet cell tumor hypoglycemia. (NICTH). These solid tumors of mesenchymal and epithelial cell origin may cause hypoglycemia.[7,8] This was confirmed by the final biopsy report of the excised mass two years prior to the onset of hypoglycemia. It was signed out as a hemangiopericytoma, a non-islet cell tumor.

HPCs are tumors that arise from pericytes, the contractile cells surrounding capillaries and venules.[9-11] The term solitary fibrous tumor is interchangeable with HPC as these have shared clinical, morphologic, immunophenotypic and genetic features.[12] Most SFTs are benign neoplasms with 12-13% rate of malignancy.[13] Of these malignant SFTs, 10 to 15% will recur and/or metastasize.[14-16] A case series reported the recurrence rate after a primary resection can be as high as 34%.[17] Once with metastases, it may cause significant symptoms such as hypoglycemia, as observed in our patient. Histologic examination of SFTs reveal contractile spindle-shaped pericytes with elongated cell processes around capillaries and post-capillary venules. These, however, may pose difficulties and diagnostic disparities due to its variable fibrous nature, protean cellularity and "pattern-less" pattern.[16,18,19] SFTs may present with symptoms and signs related to extrinsic compression of adjacent organs. In less than 5% of cases, it may lead to reactive hypoglycemia, a condition called Doege-Potter syndrome.[20-22]

Doege-Potter syndrome is a rare paraneoplastic syndrome presenting as symptomatic hypoglycemia associated with a solitary fibrous tumor.[22,23] The SFT ectopically secretes the prohormone of insulin-like growth factor II (IGF-II).[11] This prohormone suppresses growth hormone and causes decreased synthesis of IGF-binding proteins.[24] This permits increased levels of unbound and active IGF-II. When the active complexes or unbound IGF-II bind to insulin and IGF receptors in the liver and peripheral tissues, there is a subsequent decrease in the release of glucose into the circulation and increased peripheral glucose metabolism, resulting in hypoglycemia.[25]

The diagnosis requires the determination of serum IGF-I and IGF-II levels; however, these ancillary tests were not available in our country during the course of work-up of our patient. A ratio of IGF-II:IGF-I more than 10 is diagnostic of DPS.[7] Our patient's medical history, presentation, histologic and diagnostic findings of metastatic hemangio-

pericytoma and associated clinical course are sufficient for the ***presumptive*** diagnosis of Doege-Potter syndrome. There are 34 compiled cases of DPS from 1930 to 2012.[2]

The management of DPS is multi-dimensional. It includes immediate correction of hypoglycemia, reduction of the tumor burden and prevention of recurrent hypoglycemia if the tumor cannot be controlled. Some case reports have documented that surgical removal of the tumor leads to immediate resolution of hypoglycemia.[1] However, the presence of widely disseminated metastases made our patient a poor candidate for surgery. Conservatively, the initial and immediate management of hypoglycemia is through the administration of oral and/or intravenous glucose or dextrose-containing fluids when needed. In most cases, this would suffice to avoid further hypoglycemia.[2,26] Another non-invasive medical approach is to increase the volume and frequency of food intake.[27,28] Our patient was advised to have small, frequent feedings every 3 hours to prevent the recurrence of hypoglycemia.

Prednisone was also started at 30 mg once daily which alleviated the hypoglycemia. Glucocorticoids such as prednisone, typically in doses of 30 to 60 mg/day, have been most efficient for long-term remission of hypoglycemia. They provide an immediate beneficial effect by suppression of IGF-II and biochemical stimulation of gluconeogenesis and glycogenolysis.[29,30] As such, glucocorticoids can be taken indefinitely with regular (monthly) evaluation of treatment response and monitoring of side effects such as exogenous Cushing's state or adrenal insufficiency.

Neoadjuvant therapy including chemoradiation and selective embolization of tumoral feeding vessels were also reported as effective. However, these are only limited to patients with resectable masses. Since SFTs are relatively chemotherapy-resistant, there are no standard chemotherapeutic regimens in use at this time, and resection remains the treatment of choice.[31] In the setting of advanced disease where surgery could not be done or tolerated, systemic therapy may be considered an option.[30] Our patient received weekly anthracycline chemotherapy with initial improvement in glycemia. Subsequently, he deteriorated after two cycles of treatment.

Promising newer chemotherapeutic agents have been reported. A recent case series of 16 patients with unresectable extracranial metastatic hemangiopericytoma showed partial tumor response in 11 (79%) patients after the administration of temozolomide and bevacizumab. Patients under this empirically designed regimen had a median progression-free and overall survival of 9.7 and 24.3 months, respectively.[32] Pazopanib, a tyrosine kinase inhibitor that acts on vascular endothelial growth factor and platelet-derived growth factor receptors, has shown favorable effects on survival and response rates for both typical and dedifferentiated/malignant SFTs.[33-35] These new agents are not yet available in our setting.

CONCLUSION

Because surgical intervention was not a viable option, conservative medical management of the ***presumptive*** Doege-Potter syndrome from a metastatic hemangiopericytoma was challenging. Early screening for possible metastases is important in the prevention of complex sequelae that have the potential to gravely affect the patient's quality of life. We were able to draw several key learning points from our patient's case.

First, a stepwise management approach to DPS should be advocated, including essential ancillary tests, such as the measurement of IGF-I and IGF-II. Second, more aggressive and multimodal therapy may be utilized, such as trans-arterial chemoembolization, metastasectomy or trial of newer chemotherapeutic agents. Lastly, long-term follow-up and close monitoring should be emphasized to both patients and health care providers to mitigate the dismal prognosis of DPS.

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

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

Author Disclosure
The authors declared no conflict of interest.

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ARTICLE COMMENTARY

Evaluating a case report for Doege-Potter Syndrome without the confirmatory diagnostic tests, in this case a ratio of IGF-II:IGF-I > 10, is at best a ***presumptive*** diagnosis from a clinical association between a solid fibrous tumor and persistent in-hospital hypoglycemia. **Hence, due to the lack of the confirmatory test results there was initially some hesitation to publish this paper.** Nevertheless, because of the rarity of the condition and to maximize every opportunity for learning, JAFES decided to publish this case report to highlight the difficulty and limitations of endocrine practice in South East and South Asia. The challenges include the limited availability of tests in some ASEAN member countries; the prohibitive costs to the patient if the tests are sent out of the country; and the consequent delay in diagnosis.

By doing so, it is hoped that we can stimulate a discussion that will provoke member Societies to 1) Advocate and raise funds for a complete endocrine testing facility in a government or private hospital in their capital cities or regional hubs; 2) Set aside funds for this specific purpose to which endocrine training institutions can apply for financial assistance; and 3) Establish a laboratory network within the ASEAN to facilitate diagnosis of rare and interesting cases or diagnostic dilemmas that can eventually be published.

Patricia B. Gatbonton, MD, FPCP, FPSEDM
Past President, Philippine Society of Endocrinology, Diabetes and Metabolism

CASE REPORT

Journal of the ASEAN Federation of Endocrine Societies

Cryptorchidism is a Useful Clue for Idiopathic Hypogonadotropic Hypogonadism in Pituitary Stalk Thickening

Shamharini Nagaratnam,[1] Subashini Rajoo,[1] Mohamed Badrulnizam Long Bidin,[1] Norzaini Rose Mohd Zain[2]

[1]*Endocrine Unit, Department of Medicine, Hospital Kuala Lumpur, Malaysia*
[2]*Department of Radiology, Hospital Kuala Lumpur, Malaysia*

Abstract

Pituitary stalk lesions can represent a wide range of pathologies. The exact cause is often unknown due to hesitancy to proceed with biopsy. We present a 16-year-old adolescent who presented with delayed puberty, short stature and bilateral cryptorchidism. He was found to have a thickened pituitary stalk of uncertain etiology with partial hypopituitarism (gonadotrophin and growth hormone deficiency) on further assessment. The presence of bilateral cryptorchidism and micropenis represents lack of "mini puberty," a phenomenon of activation of the hypothalamic-pituitary-gonadal (HPG) axis in-utero or within the first few months of life.[1] These key clinical features have been useful to establish an early temporal relationship and suggest a congenital origin of disease. This enabled a more conservative approach of surveillance to be employed as opposed to invasive pathological examination with pituitary stalk biopsy.

Key words: pituitary disease, hypopituitarism, cryptorchidism, growth hormone, hypogonadotrophic hypogonadism

INTRODUCTION

Pituitary stalk lesions comprise a broad spectrum of diseases that impose a great challenge to the treating physician due to its critical location and pivotal role in the hypothalamic-pituitary (HPA) axis. This proves more of a conundrum in children and adolescents whereby a prompt diagnosis is pertinent to avoid long term repercussions of disease progression and hypopituitarism. The presence of hypopituitarism associated with thickened pituitary stalk implies compromise to the function of the gland which may be permanent or reversible in some cases depending on the etiology and duration of disease. The exact etiology of these lesions often unknown despite rigorous work up, typically warranting a pituitary stalk biopsy as the next step of management. In many cases, there is reluctance to proceed with pathological examination due to the risk of complications. Therefore, the presence of specific clinical features in addition to radiological and laboratory investigations is critical to establish the temporal relationship of insult to the HPA axis and determine possible origin of disease. Bilateral cryptorchidism and micropenis which represents lack of 'mini puberty' are key features supporting an early insult to the hypothalamic-pituitary-gonadal (HPG) axis in patients with hypogonadotrophic hypogonadism.[1,2]

CASE

We present a 16-year-old boy referred from urology for further evaluation of hypogonadotrophic hypogonadism. He was initially diagnosed with bilateral cryptorchidism aged 2, and his parents opted for conservative management. There was no spontaneous descent of testes in the next few years and he was eventually lost to follow up. He was referred again to urology aged 12, for recurrent urinary tract infections and was diagnosed with vesico-ureteric reflux (VUR) warranting bilateral ureteric stenting and implantation. There were no other structural or functional abnormalities of the renal system identified. Cryptorchidism was reevaluated at this point and he underwent bilateral orchidopexy, with successful descent only on the left side. Routine clinical and hormonal assessment done at this juncture revealed hypogonadotrophic hypogonadism, warranting an endocrine consult.

Detailed history taking from parents and physical examination elicited the following salient clinical features. He was born full-term with an uneventful antenatal history. His height and weight remained within the 25th and 50th centile till the age of 10. A decline in growth rate was noticed as he approached adolescence, whereby he was one of the shortest amongst his peers in secondary school. He had been a slow learner, with poor academic performance since primary school. His parents had noticed him having low energy levels and fatiguability in the later years. No history of anosmia or hearing deficits was elicited. He had no history of polyuria or polydipsia to suggest cranial diabetes insipidus. A full systemic review did not reveal symptoms to suggest intra-cranial mass effect such as chronic headaches, visual disturbances or neurological deficits. He had no past history of intracranial pathology, trauma or radiation. Apart from the history of VUR and cryptorchidism, he did not suffer from any chronic diseases nor receive long term immunosuppression/steroids or chemotherapy. He has no family history of short stature nor delayed puberty. He has one younger sibling, a 12-year-old female who has achieved puberty and is of appropriate height. His anthropometric measurements of weight and height were charted as 58 kg (between 5-10th

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Corresponding author: Shamharini Nagaratnam, MB BCh Bao, MRCP(UK)
Endocrine Fellow, Endocrine Unit,
Department of Medicine, Hospital Kuala Lumpur,
Jalan Pahang, 50586 Kuala Lumpur, Malaysia
E-mail: shamharininagaratnam@yahoo.com
ORCiD: https://orcid.org/0000-0002-1505-3397

centile), 155 cm (below 5th centile) respectively. His height was significantly lower than his mid-parental height of 170 cm. On physical examination, he had no midline facial deformities to suggest a syndromic constellation. Secondary sexual characteristics were also absent. Tanner staging for pubic hair and genitalia was pre pubertal (stage 1) with absent pubic hair, micropenis, minimal hyperpigmentation with no ruggae over the scrotum and testicular volume measuring approximately 1 ml (left) and absent (right).

His anterior pituitary hormone panel showed hypogonadotropic hypogonadism with low FSH, low LH and undetectable testosterone levels. Insulin- like growth factor 1 (IGF-1) levels was found to be lower than expected for his age and gender raising the suspicion of concomitant growth hormone (GH) deficiency. His other pituitary hormones were intact. (Table 1). Insulin Tolerance Test (ITT) with testosterone priming was carried out to confirm growth hormone deficiency. This test was inconclusive due to failure to achieve adequate hypoglycemia.

Table 1. Anterior pituitary hormone panel

Hormone	Result		Reference range	
FSH	2.2 IU/L		1.5-12.9	
LH	0.58 IU/L		1.3-9.8	
Testosterone	<0.1 nmol/L		<28.8	
IGF-1	273 ug/L		267-673	
Prolactin	196.8 mIU/L		86-324	
Am cortisol	569.9 nmol/L		145.4-619.4	
Thyroid function test	TSH	2.94 mIU/L	TSH	0.52-4.30
	Free T4	15.76 pmol/L	T4	12.8-21.0

As an alternative test, Glucagon Stimulation Test (GST) was chosen. GST showed a peak GH of 6.94 ng/ml (>10 ng/ml) at 90 mins confirming his growth hormone deficiency.[3] Bone age assessment showed his skeletal age to lag at the age of 12-13 years with unfused epiphyseal plates. A MRI pituitary ordered showed an enlarged pituitary stalk extending to the tuber cinereum with homogenous enhancement post gadolinium contrast. The stalk measured 13 mm (AP diameter) at the point of insertion at the infundibulum with uniformed pattern of thickening. The pituitary gland itself was not hypoplastic, with no focal enhancement on post contrast study. The optic nerve, chiasm and tract was not thickened, with no evidence of compression. (Figures 1 and 2). Therefore, at this point it was concluded that this patient had thickened pituitary stalk with partial hypopituitarism (hypogonadotropic hypogonadism and growth hormone deficiency).

The etiology of thickened pituitary stalk was worked up extensively involving laboratory and radiological investigations. His complete blood count showed no evidence of hematological dyscrasias. His liver and renal biochemistry was normal. Both inflammatory and tumor markers were not raised. There was no evidence of mediastinal or lung mass on chest x-ray. Ultrasound showed very small testes in the right inguinal region and left scrotum measuring 1.2-1.5 cm, both testes had no suspicious malignant features or associated lymphadenopathy. In summary, there were no red flags to suggest an inflammatory or neoplastic process. A multidisciplinary discussion was held to discuss the role of pituitary stalk biopsy for him. The presence of pre-pubertal features

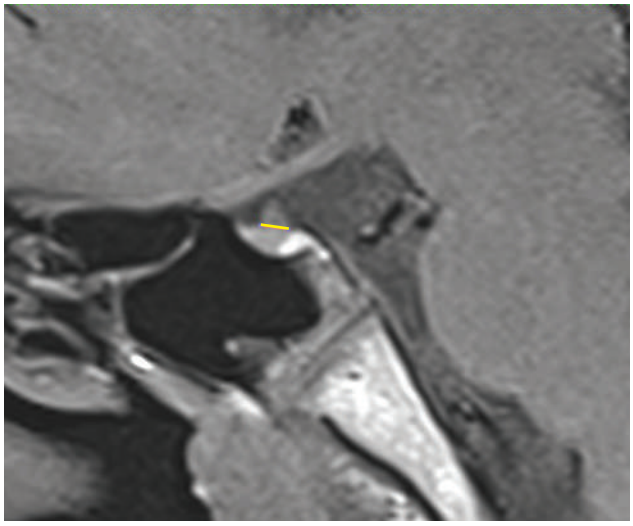

Figure 1. Sagittal T1WI MRI Pituitary showing enlargement of the pituitary stalk, measuring 13 mm in AP diameter.

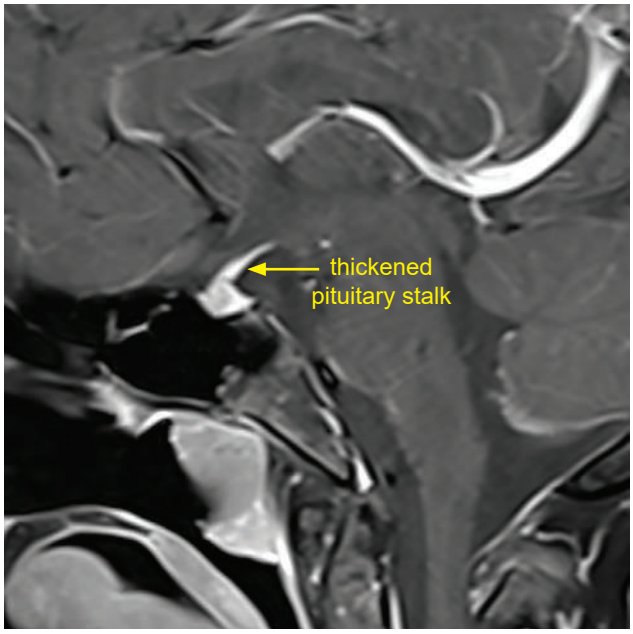

Figure 2. Post gadolinium sagittal (T1WI) MRI of pituitary showing homogenous enhancement of the pituitary stalk (yellow arrow). The pituitary gland is small in size and has no focal lesion.

representing gonadotroph deficiency early in life coupled with non-suspicious laboratory and imaging workup was highly suggestive of congenital origin of disease. Therefore, a decision was made not to proceed with pituitary stalk biopsy unless there is evidence of disease extension or progressive loss of pituitary function.

Growth hormone, norditrophin at a dose of 0.025 mcg/kg/day was initiated in this boy aiming to achieve near adult height. He showed good response to therapy with a 4 cm increment in height after 3 months. Puberty induction will be initiated once acceptable near adult height is achieved. Serial MRI pituitary and pituitary hormone panel will be repeated every 6 months to monitor progression of disease. He is also planned for removal of the right testis due to risk of malignancy and poor function.

DISCUSSION

The etiology of thickened pituitary stalks is often broadly divided into 3 categories: neoplastic, inflammatory or infectious and congenital.[4] Two large case series based on retrospective review involving both adults and children with pituitary stalk lesions have showed neoplastic lesions to be the leading cause of pathology.[4,5] A similar finding was found in a study done in Korea, specifically in the pediatric population below the age of 18 with pituitary stalk lesions.[6] However, Turcu et al., also found 39% of pituitary stalk lesions in a retrospective review with unclear pathology.[4] The role of pituitary stalk biopsy often remains controversial due to the risk involved. Despite using a minimally invasive endoscopic approach, the procedure, coupled with manipulation of the pituitary stalk, imposes a risk of cranial diabetes insipidus, hypopituitarism, cerebrospinal fluid (CSF) leak and meningitis in patients. In addition, there is approximately a 10 % risk of a negative biopsy, whereby a diagnosis is not histologically conclusive despite adequate tissue sample.[7]

Radiological features on MRI is often the key in guiding the diagnosis of pituitary stalk lesions. It is also an essential tool to monitor progression of disease or response to treatment in these patients. Specific patterns of enhancement such as uniform, V-shaped, round or diamond, and pyramidal has been associated with various pathologies in literature. The strongest association found was between the congenital lesions and round pattern of enhancement in the Mayo experience by Turcu et al. Extent of lesions was also associated with hypopituitarism, and the lesions with hypothalamic extension have been found to have the highest risk of hypopituitarism.[4]

Congenital cryptorchidism is one of the most common congenital malformation in boys. Its prevalence at birth among boys with a birth weight more than 2,500 g has been reported to range between 1.8% and 8.4%. It is associated with reduced concentration of testosterone and sperm quality in adulthood. There are numerous causes for congenital cryptorchidism including disorders involving sex chromosomes, gonadal development, decrease in androgen synthesis and action, structural defects and hypogonadotrophic hypogonadism.

Typically, pituitary gonadotropes start producing FSH and LH around 9 weeks of gestation, reaching their peaks at mid-gestation with a concomitant rise in testosterone. This peak of testosterone is essential for descent of testes in utero. The activation of hypothalmic-pituitary-gonadal (HPG) axis occurs approximately 1 week after birth with a second peak at 1–3 months, a period also synonymous with 'mini-puberty.' This post- natal surge is essential for testicular descent and penile growth. In those with congenital hypogonadotropic hypogonadism, the lack of gonadotrophins results in the arrest of testicular descent and penile growth in utero and in the neonatal period.[2]

CONCLUSION

In our patient, the lack of 'mini puberty' described above suggests the insult to the HPG axis occurred early in life, pointing towards a congenital origin of disease. Despite the radiological features not being typically described in literature for congenital lesions, the extension of the lesion to the hypothalamus accounts for the development of partial hypopituitarism in this patient. In addition, the negative results for both inflammatory and neoplasm workup further supports this diagnosis. With congenital disease as our probable diagnosis, we have chosen a more conservative approach of management with surveillance as described above, alleviating the need for pituitary stalk biopsy at the moment.

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

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

Author Disclosure
The authors declared no conflict of interest.

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CASE REPORT

Ectopic Cushing's Syndrome secondary to Recurrent Thymic Neuroendocrine Carcinoma with Bilateral Ovarian Metastases: A Case Report

Eunice Yi Chwen Lau and Zanariah Hussein

Division of Endocrinology, Department of Medicine, Hospital Putrajaya, Federal Territory of Putrajaya, Malaysia

Abstract

Cushing's syndrome due to ectopic adrenocorticotrophic hormone (ACTH) secretion is uncommon, accounting for 9 to 18% of cases; approximately 10% of ACTH producing tumours are caused by thymic carcinomas.[1] We describe a young lady who presented with Cushing's syndrome secondary to a primary neuroendocrine tumour (NET) arising from the thymus. She had surgical resection of her primary tumour with remission of her Cushing's syndrome however subsequently went on to have locoregional recurrence followed by distant metastases to her bilateral ovaries. She underwent 6 surgeries including bilateral adrenalectomy and had 3 cycles of chemotherapy over the course of the 8 years since her diagnosis. Due to the rarity and highly aggressive nature of this disease, we highlight the need for a multidisciplinary team approach and use of multiple modalities in the management of our patient. Timely use of bilateral adrenalectomy particularly in young patients is important to prevent further complications and facilitate other treatment modalities.

Key words: adrenalectomy, ectopic ACTH syndrome, Krukenberg tumour, neuroendocrine tumour, thymic tumour

INTRODUCTION

Thymic neuroendocrine tumours (TNETs) are rare tumours with an incidence of 0.02 per 100,000 population per year among Caucasians and 0.04 per 100,000 population per year among Asian or Pacific Islanders based on data from the SEER database.[2] In the latest 2015 World Health Organisation classification, TNETs have been classified into 4 categories: Typical carcinoids (TC) (low grade or grade 1) and atypical carcinoids (AC) (intermediate grade or grade 2) which are well differentiated; large and small cell neuroendocrine carcinomas (high grade or grade 3) which are poorly differentiated.[3] Data have suggested that Asian patients may have a predominance of AC with one study in Beijing showing AC in 60%, TC in 10% and 27% were poorly differentiated. By comparison, the SEER database which comprised of 83% Caucasians, only 10% of patients were AC.[4] Diagnosis may be challenging as one-third of patients are asymptomatic and are only identified on routine imaging. Others may present with mass effect due to compression of surrounding nerves or vessels. Carcinoid syndrome is rare, occurring in less than 1% of TNETS while approximately 40 to 50% can present with ectopic ACTH syndrome.[4] Twenty five percent of patients are associated with multiple endocrine neoplasia 1 (MEN-1) syndrome.[4] As this is a rare condition, there is a lack of prospective trials or large series and cases are managed in a similar manner as broncho-pulmonary carcinoids. Ovarian metastases can occur in neuroendocrine tumours (NETs) but they are uncommon and there is no current recommendation on their management in this situation. In this case report, we present a complex case of a young female with recurrent metastatic thymic neuroendocrine tumour with bilateral ovarian metastases and the multiple treatment modalities that were used over the years for her disease control.

CASE

A 23-year-old female of Indian ethnicity presented with hyperpigmentation, acne, progressive weight gain and irregular menses over 3 years. Clinically, she appeared cushingoid with truncal obesity and moon facies. She achieved menarche at age 13 and previous menses were regular. She had no history of past medical illness and no family history suggestive of MEN-1 syndrome. Her initial investigations performed at a private medical centre showed a markedly elevated adrenocorticotrophic hormone (ACTH) level of 221 mIU/L and positive high dose dexamethasone suppression test. Computed tomography of the thorax, abdomen and pelvis (CTTAP) showed an anterior mediastinal mass measuring 2.1x2.8x3.0 cm, located behind the sternum and hyperplasia of the adrenal glands. She underwent surgical removal of her anterior mediastinal mass by a cardiothoracic surgeon at a private centre. Histopathology showed a moderately differentiated neuroendocrine tumour grade 2 with a mitotic index 5/10 hpf and Ki-67

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Corresponding author: Eunice Yi Chwen Lau, MD
Endocrinology Fellow-in-Training / Physician
Putrajaya Hospital, Presint 7,
62250 Federal Territory of Putrajaya, Malaysia
Tel. No.: (60)3-83124200
Fax No.:(60)3-88880137
E-mail: eunique_lyc@yahoo.com
ORCiD: https://orcid.org/0000-0003-2425-2096

proliferative index of 10%. The tumour stained positive for synaptophysin, chromogranin and ACTH and was negative for thyroid transcription factor-1 (TTF-1). Post-operatively, she displayed clinical resolution of her Cushing's syndrome with resumption of her regular menses and a Gallium-68 (Ga-68) DOTATOC positron emission tomography (PET)/CT scan 4 months post-operatively showed no evidence of somatostatin receptor (SSTR) avid disease.

A year post-surgery, she developed progressive weight gain and increasing ACTH levels. CTTAP showed recurrence with multiple solid nodules at the site of her previous surgery in the upper left mediastinum. Ga-68 DOTATOC PET/CT scan showed new focus of SSTR avid disease in the superior mediastinal node anterior to the innominate artery and multiple pre-vascular nodes near the previous surgical site. F18-Fluorodeoxyglucose (F18-FDG) PET/CT scan showed concordant uptake with the Ga-68 DOTATOC PET/CT scan. She had her second surgery approximately 3 years after her first surgery, with complete removal of her mediastinal tumour. Histopathology was consistent with an intermediate grade metastatic neuroendocrine tumour.

Her symptoms recurred a year after her second surgery. She developed diabetes and hypertension requiring medical therapy and was commenced on ketoconazole. CTTAP imaging showed an enlarged left supraclavicular node measuring 1.2 cm in the short axis diameter and reduction in size of her mediastinal lesion measuring 1.3x2cm. Ga-68 DOTANOC PET/CT showed new focus of uptake at the left supraclavicular node with resolution of previous uptake at the superior mediastinum and pre-vascular nodes. Subsequently, 4½ years after her first surgery, an attempt was made to remove the left supraclavicular lymph node. However, adhesions to the surrounding structures resulted in inability to completely remove the node.

Post-operatively, she had persistent Cushing's syndrome. Metyrapone was added to her therapy, nevertheless she continued to have difficult to control diabetes, hypokalaemia and progressive cushingoid features. CTTAP imaging approximately 1½ years after her 3rd surgery showed multiple rounded lobulated lesions along the right postero-lateral uterine body largest measuring 4.6x4.4x5.2 cm. There was no significant change in the left supraclavicular lesion. F18-FDG PET/CT scan showed uptake in both the supraclavicular and pelvic lesion, while Ga-68 DOTANOC PET/CT scan showed uptake in the supraclavicular lesion and right level II cervical nodes and anterior mediastinal node.

At this point decision was made for bilateral adrenalectomy (5½ years after first surgery) for management of her hypercortisolism. Prior to adrenalectomy, she required 3000 mg of metyrapone, 400 mg ketoconazole, 200 mg spironolactone, 160 mg valsartan, 10 mg amlodipine, 2000 mg metformin, 120 mg of modified release gliclazide, 10 U of intermediate acting insulin, 20 mg atorvastatin, 1200 mg potassium chloride and 1000 IU cholecalciferol per day.

Post-operatively, she had resolution of her Cushing's features, was able to stop all her potassium supplements, antihypertensive and antidiabetic medications and was put on glucocorticoid and mineralocorticoid replacement.

However, she continued to have increasing hyperpigmentation (Figure 1) and CTTAP imaging surveillance 6 months after her surgery showed increase in size of her uterine lesion likely right adnexal in origin measuring 5.8x5.2x5.2 cm in size (Figure 2A). Ga-68 DOTANOC PET/CT and F18-FDG PET/CT imaging were performed showing predominantly FDG avid disease (Figure 2B and 2C), hence she was deemed unsuitable for peptide receptor radionuclide therapy (PRRT). Following multidisciplinary team discussion, she was recommended for surgery to remove the adnexal lesion. She was offered bilateral salphingoopherectomy and hysterectomy and the implications were discussed in terms of long-term gonadal function and bone health. She opted for a more conservative approach and underwent laparotomy, right salphingoopherectomy, left cystectomy, omentectomy and appendectomy.

Intra-operative findings showed a right large ovarian tumour measuring 10x10 cm adherent to the right pelvic wall and 2 cysts in the left ovary measuring 1x1 cm and 2x2 cm (Figure 3A). Histopathology showed high grade metastatic neuroendocrine carcinoma with a Ki-67 proliferative index of 10%, mitotic figures of >10/2 mm^2 and presence of capsular breach, lymphovascular invasion in both right and left ovaries. Marked improvement in ACTH levels were noted after surgery (Figure 4). Prior to surgery she continued to have her normal periods but was amenorrhoeic after. She was also given 3 cycles of chemotherapy with etopside (100 mg/m^2 Day 1 to 3) and cisplatin (25 mg/m^2 Day 1 to 3). However, CTTAP at 6 months showed progression of her left adnexal mass and ACTH levels continued to increase (Figure 4). She went on to have extrafascial hysterectomy and left salphingoopherectomy 8 months after the 5th surgery. Intra-operative findings showed a 6-week size uterus, a 3x3 cm left ovarian tumour with intact capsule and a ruptured right sided pelvic wall tumour measuring 5x4 cm (Figure 3B). Left ovarian histopathology was consistent with a high-grade neuroendocrine tumour (mitosis >10/2 mm^2).

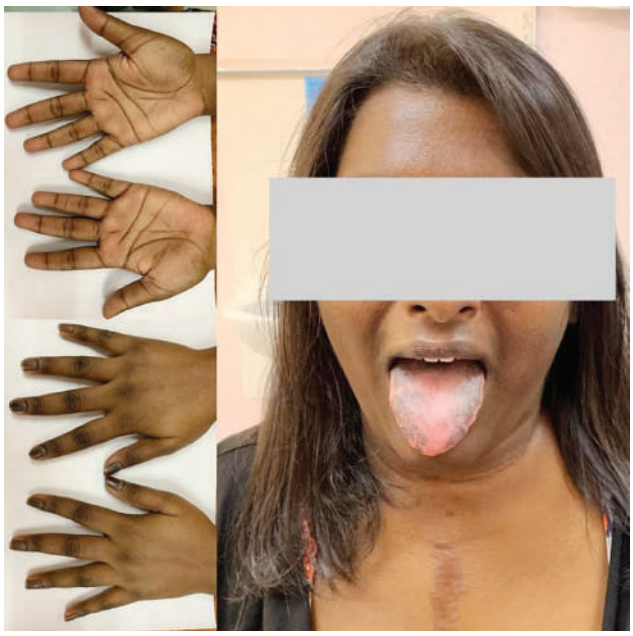

Figure 1. ACTH induced hyperpigmentation.

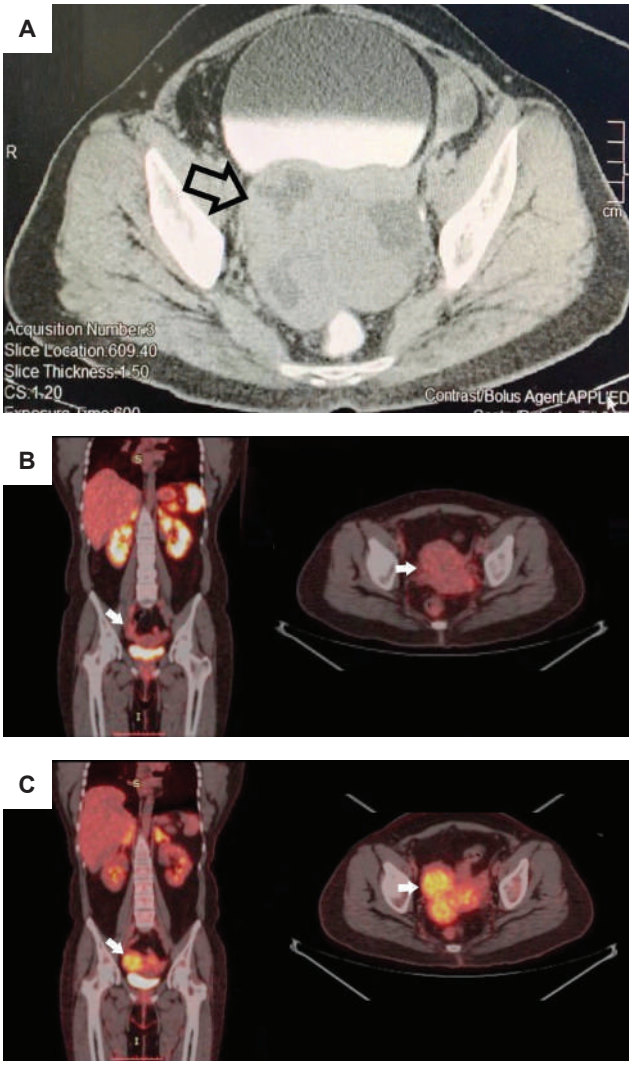

Figure 2. (A) CT image showing heterogenous multilobulated pelvic lesion; **(B)** Ga-68 DOTANOC PET-CT showing pelvic lesion with no SSTR avid disease. **(C)** FDG PET-CT showing FDG hypermetabolism of the pelvic lesion.

After surgery, her ACTH levels reduced to 49.5pmol/L and the trend is demonstrated in Figure 4. She developed symptoms of flushing and fatigue despite adequate glucocorticoid and mineralocorticoid replacement and was started on conjugated oestrogen 0.625 mg and medroxyprogesterone 5 mg daily. She is currently still followed-up at our centre and remains positive with good family support.

DISCUSSION

TNETs consist of 2% of all mediastinal tumours and 5% of all thymic tumours, making ectopic ACTH secreting TNETs exceedingly rare. TNETs usually present around the 5th decade and have a male predominance.[2,5] Comparatively, TNETs associated with ectopic ACTH syndrome appear to present younger, around 21 to 35 years of age with hyperpigmentation being a prominent feature.[6,7] They also appear to have poorer outcomes, although it is uncertain whether this is due primarily to the metabolic complications associated with Cushing's syndrome or more aggressive tumour behaviour.[6]

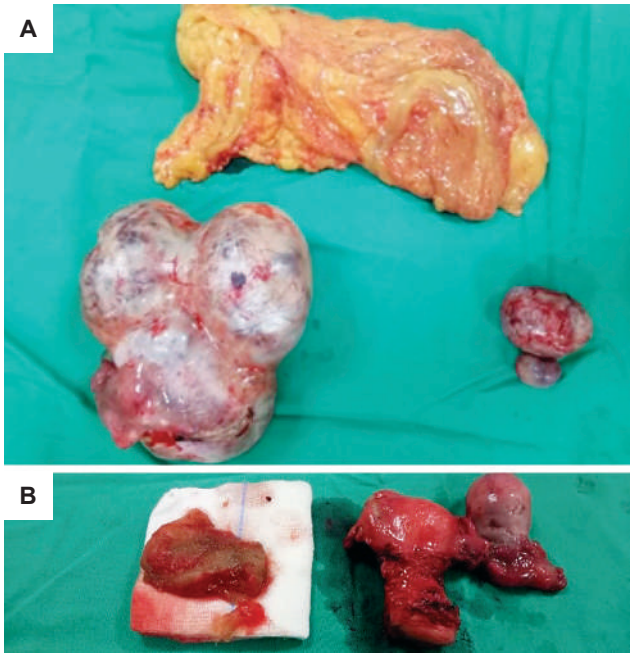

Figure 3. (A) Large right ovarian tumour, 2 left ovarian cysts and omentum; **(B)** Ruptured right pelvic tumour, uterus with left ovarian tumour.

A recent study of ectopic ACTH secreting TNETs in China showed median overall survival of 41 months and progression free survival of 28 months with significant improvement after complete resection.[7] There appears to be wide heterogeneity in the tumours, while some may be rapidly progressive, others are more indolent and may recur up to 8 years after surgery.[5,6]

Our patient achieved clinical and biochemical remission after initial tumour resection. However, her disease subsequently progressed over the period of several years requiring repeat surgery followed by adrenalectomy when the tumour became unresectable. Ovarian metastases while uncommon, can occur in TNETs as shown in this case. Most of the treatment decisions for this patient were made after multidisciplinary discussion with oncologists, surgeons, endocrinologists, radiologists, and gynaecologists. As there are no clear guidelines on the management of this disease, a multidisciplinary approach is important, and we highlight the multiple treatment modalities and review the evidence for their use.

In the past TNETS have been managed in a similar manner to bronchopulmonary carcinoids and were classified and graded as a single class of tumours. However, in recent years they have been acknowledged as having their own distinct behaviour and genetic background. Unlike their lung counterparts, TNETs have a more aggressive clinical course and frequently metastasize to lymph nodes, bone and lung.[8,9] Less commonly, metastases have been reported in widespread locations including the oesophagus, chest wall, liver, brain, pancreas, kidney and adrenal gland.[8,9]

In our patient, the tumour metastasized to both ovaries. This case is unique as our patient had bilateral ovarian metastases from a TNET which showed evidence of ACTH production. There has been report of ACTH secreting

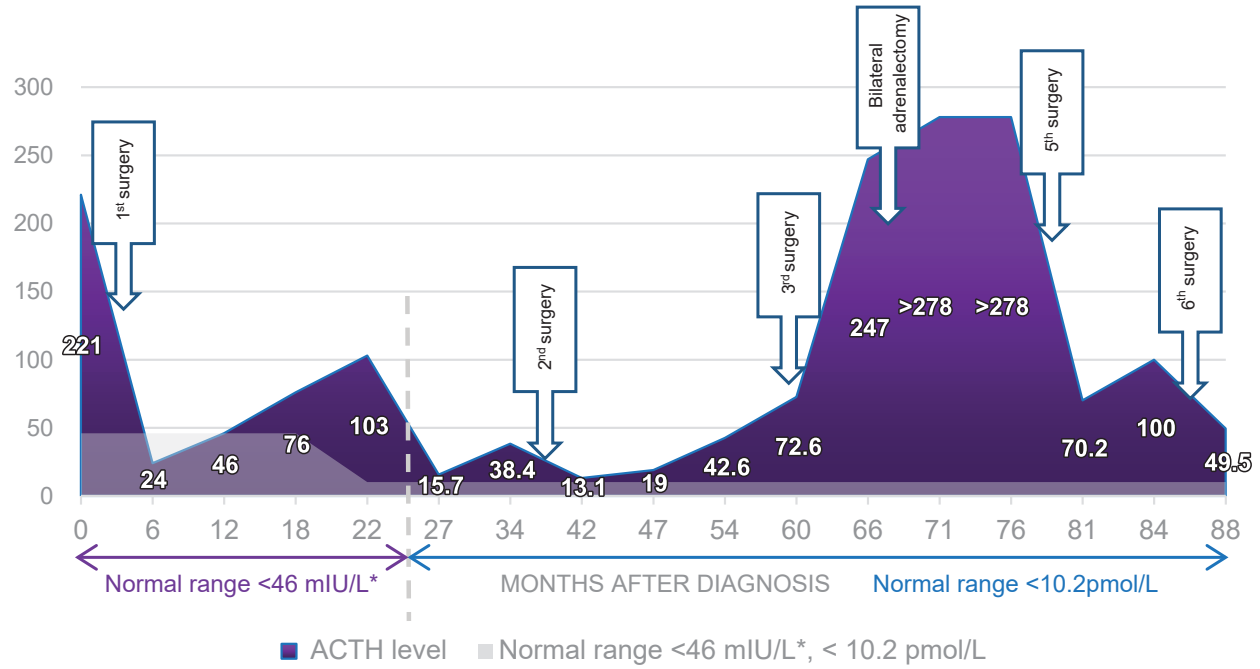

Figure 4. ACTH trend since diagnosis and corresponding normal range. Difference in ACTH cut-offs before and after 22 months was due to a change in assay used.

pancreatic NET presenting with bilateral ovarian metastasis but to our knowledge there is no published report of a functioning thymic NET with ovarian metastases.[10] Generally thymic malignancies metastasize to intrathoracic sites first, with abdominal sites being involved later during the disease.[11] Liver metastasis were the most common site of extra-thoracic metastasis with ovarian metastasis being rare.[11] In neuroendocrine tumours, ovarian metastases were estimated to occur in approximately 2% patients and usually originated from midgut tumours predominantly the small bowel.[12,13] They were usually bilateral, unlike primary ovarian lesions which were unilateral and could range from microscopic foci up to large tumours of 9.5 cm like our case.[13] Previous reports have suggested that patients usually had well-differentiated NETs and 76% had synchronous liver and peritoneal metastases (including mesentery and omentum).[13]

Radical surgery has been widely agreed as the only option for cure. However, this may not be feasible in all patients as more than 50% have locally advanced disease or metastases at presentation. Even after radical surgery, recurrence is common as demonstrated by our case. Studies have shown that the most important independent prognostic factor is the completeness of resection whereby those who achieved R0 resection had improved overall survival compared to those who did not.[5,7] Early stage tumours have also been reported to survive longer and have less recurrence.[5] On the other hand, tumour histology did not appear to have significant prognostic implication. For recurrent or metastatic disease surgery may be considered if feasible. For primary ovarian NETs prophylactic omentectomy and hysterectomy have been performed as adapted from the management of ovarian cancer although it is uncertain whether this provides any additional benefit.[14] Our patient was initially offered bilateral salphingoopherectomy and hysterectomy however she opted for a more conservative approach. Despite adjuvant chemotherapy, her disease continued to progress, and she subsequently required repeat surgery. This led to improvement in her ACTH levels. During this surgery, it was noted that the pelvic tumour had ruptured, likely leading to intraperitoneal seeding. This has been shown to greatly increase the risk of relapse in epithelial ovarian cancers.[15] Hence, she will require careful monitoring for relapse with ACTH levels and imaging as well as long-term female gonadal hormone replacement on top of her adrenal hormone replacement.

For tumours that are metastatic or unresectable chemotherapy and radiotherapy may play a role although clear evidence is lacking. One study showed possible benefit of adjuvant therapy on progression free survival but not for overall survival[7] while another study failed to show any benefit at all[5] as was the case in our patient. Patients that require adjuvant therapy probably had poorer prognosis and more extensive disease hence more research is needed to clearly evaluate this area.

In our patient, there was also difficulty in managing the metabolic complications of her Cushing's syndrome. Similar to previous reports, tumour recurrence was associated with re-development of Cushing's syndrome.[6] Management of hypercortisolism is important and can be managed medically with drugs such as ketoconazole, metyrapone and less commonly mitotane in view of its serious side effects. In ectopic ACTH secreting neuroendocrine tumours, bilateral adrenalectomy is usually reserved for situations as in our patient whereby the primary tumour cannot be completely removed, have local recurrence or metastatic disease. The other instance would be when the primary tumour is occult and remains unidentified during follow-up. One study showed improvement in metabolic and adverse events (infection, fracture, thrombosis) scores in patients with refractory ACTH dependant Cushing's

who underwent bilateral adrenalectomy compared to those who used steroidogenesis inhibitors alone.[16] Hence, early bilateral adrenalectomy should be considered in those with reasonable life expectancy from their primary tumour as this may facilitate surgical resection or other treatment modalities in initially unresectable tumours and prevent further adverse complications.

CONCLUSION

ACTH producing TNET is an aggressive disease, and a multidisciplinary team approach is needed. First-line treatment is surgery, especially in young patients. There is also the challenge of uncontrolled Cushing's and early adrenalectomy may be considered to optimize management. Young patients may have a prolonged disease course like our patient, requiring multiple treatment modalities as the disease progresses.

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

Statement of Authorship
Both authors certified fulfilment of ICMJE authorship criteria.

Author Disclosure
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CASE SERIES

Giant Parathyroid Adenoma versus Parathyroid Carcinoma: Differentiating Two Entities

Hazwani Aziz[1] and Zanariah Hussein[2]

[1]*Hospital Tuanku Jaafar, Ministry of Health Malaysia*
[2]*Hospital Putrajaya, Ministry of Health Malaysia*

Abstract

Giant parathyroid adenoma (GPA) is defined as adenoma larger than 3.5 g. Twenty-one cases of parathyroid mass >3.5 g in patients with primary hyperparathyroidism who underwent parathyroidectomy in Hospital Putrajaya, Malaysia were identified. Most cases presented with nephrolithiasis. Two cases are reported as parathyroid cancer. GPA has significantly higher serum calcium and iPTH levels and can be asymptomatic. Parathyroid carcinoma patients are frequently symptomatic, with large tumors. Differentiating GPA from parathyroid cancer is important as it determines the subsequent surgical intervention.

Key words: hyperparathyroidism, primary, parathyroid neoplasm, parathyroidectomy, calcium, adenoma

INTRODUCTION

Primary hyperparathyroidism is a common endocrine disorder with a reported incidence of 25 per 100,000 in the general population.[1] Only less than 1% of cases of primary hyperparathyroidism are due to parathyroid cancer, and 85% are due to parathyroid adenoma.[2] Giant parathyroid adenoma is a rare condition as parathyroid adenomas are commonly reported as small lesions, weighing less than 1 gram.[1] Giant parathyroid adenoma is defined as an adenoma of more than 3.5 g weight.[3] There has been no reported prevalence data of giant parathyroid adenoma among ASEAN countries. This case series adds to the body of knowledge generally lacking in the incidence of parathyroid disease in the Asian population.

This case series describes the different demographics, clinical presentations, laboratory data, tumor sizes and histopathological reports of patients who underwent parathyroidectomy in Hospital Putrajaya, Malaysia with parathyroid mass larger than 3.5 g.

CASE SERIES

Twenty-one cases of parathyroid mass larger than 3.5 g in patients with primary hyperparathyroidism who underwent parathyroidectomy in Hospital Putrajaya, Malaysia from 2012 till 2019 were identified from the electronic medical records. A total of 87 patients with primary hyperparathyroidism were collected and 21 of the patients (24%) had large parathyroid mass. The majority of patients were male (62%), and between 50 to 65 years old (62%) at presentation (Table 1).

Eleven cases (52%) presented with nephrolithiasis. Six cases had osteoporosis and two were asymptomatic. A case of severe parathyroid bone disease with osteoporotic fracture occurred in a 17-year-old female with markedly elevated serum parathyroid hormones (iPTH) of 89.8 pmol/L. Two cases presented with pancreatitis and severe hypercalcaemia of 3.21 mmol/L and 4.0 mmol/L.

Average serum calcium at presentation was 3.2 mmol/L, with only 2 cases having serum calcium levels of less than 3.0mmol/L. Average iPTH level was 71.5 pmol/L, with highest iPTH level of 176.6 pmol/L. The tumor sizes ranged from 3.5 g to 38 g.

Two cases developed hungry bone syndrome post-surgery. The first case was a patient with an adenoma size of 32.4 g, with serum calcium of 3.14 mmol/L and serum alkaline phosphatase (ALP) of 3046 U/L at presentation. The second case was a patient with an adenoma size of 6 g, and serum calcium of 3.22 mmol/L and serum ALP of 405 U/L.

Upon histopathological review, two cases were reported as parathyroid cancer and a single case was classified with atypical histology suggestive of cancer. Both had significantly high iPTH levels.

The first case was a 28-year-old female, presenting with nephrocalcinosis and chronic pancreatitis, with serum calcium of 4.0 mmol/L, iPTH level of 176 pmol/L and tumor size of 4.2 g. The second case was a 78-year-old female who presented with symptomatic hypercalcemia and osteoporosis, with serum calcium of 3.61 mmol/L, iPTH level of 88.2 pmol/L and tumor size of 38 g. A case

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Corresponding author: Hazwani Aziz, MMed (Int Med) UKM
Hospital Tuanku Jaafar, Jalan Rasah, Bukit Rasah, 703000,
Seremban, Negeri Sembilan, Malaysia
Tel. No.: +6067684000
Fax No.: +6067625771
E-mail: wanie82girl@yahoo.co.uk
ORCiD: https://orcid.org/0000-0001-6741-314X

* *The paper has been presented as poster presentation during AFES 2019 in Philippines on 21-23 November 2019.*

Table 1. The summarized relevant data of all the patients with parathyroid mass of more than 3.5 g, with relevant laboratory investigations; serum calcium, serum inorganic phosphate, serum alkaline phosphatase (ALP), serum parathyroid hormone (iPTH), their clinical presentations, associated syndromes and the results of their biopsies

Case	Age/ Gender	Calcium (mmol/L)	Inorganic Phosphate (mmol/L)	ALP (U/L)	iPTH (pmol/L)	Clinical presentations/ associated syndromes	Weight (g)	HPE
1	50/M	3.70	0.81	101	67.2	Nephrolithiasis	14.2	Adenoma
2	35/M	3.05	0.90	78	16.8	Recurrent PHPT Nephrolithiasis Osteoporosis	4.3	Adenoma
3	61/M	3.34	0.81	179	113.5	Assymptomatic	9.1	Adenoma
4	57/M	3.26	0.85	77	37.1	Nephrolothiasis	4.9	Adenoma
5	63/M	4.23	0.90	95	67.0	Nephrolithiasis	5.8	Adenoma
6	53/F	3.21	0.69	139	34.1	Pancreatitis Osteoporosis	10.1	Adenoma
7	63/F	3.26	1.00	80	83.5	Constipation Nephrolithiasis	8.7	Adenoma
8	53/F	3.85	0.64	194	99.2	Osteoporosis Nephrolithiasis/MEN1	15.6	Adenoma
9	48/M	3.06	0.68	171	28.5	Nephrolithiasis	3.7	Adenoma
10	63/F	3.00	0.51	204	50.3	Nephrolithiasis	5.3	Adenoma
11	28/F	4.00	0.69	173	176.0	Nephrolithiasis Pancreatitis	4.2	Carcinoma
12	58/M	3.22	0.56	405	40.4	Polyuria Osteoporosis	6.0	Adenoma
13	37/M	3.00	0.89	73	36.5	Constipation Nephrolithiasis/MEN1	4.1	Hyperplasia
14	60/M	3.59	0.60	142	40.8	Confusion	5.8	Adenoma
15	17/F	3.14	0.40	3046	89.8	Fractures Osteoporosis	32.4	Adenoma
16	24/M	2.60	0.60	142	63.7	Abdominal pain	3.9 /1.0	Hyperplasia
17	51/M	3.03	0.57	90	56.8	Nephrolithiasis	3.5	Adenoma
18	52/F	2.77	0.50	1396	87.8	Constipation Bone pain	6.9	Atypical adenoma
19	59/M	3.21	0.56	143	41.2	Assymptomatic	7.6	Adenoma
20	39/M	4.64	0.66	388	176.6	Lethargy Polyuria	14.6	Adenoma
21	78/F	3.61	0.67	146	88.2	Confusion Osteoporosis	38.0	Carcinoma

Abbreviations: M, male; F, female; ALP, alkaline phosphatase; iPTH, intact parathyroid hormones; HPE, histopathological examination; MEN, multiple endocrine neoplasia.

of atypical parathyroid adenoma with focal capsular and perivascular invasion occurred in a 52-year-old female with serum calcium of 2.77 mmol/L, serum iPTH of 87.8 pmol/L and tumour size of 6.9 g.

Two cases were associated with Multiple Endocrine Neoplasm 1 (MEN1), each showed histopathological features of parathyroid hyperplasia with tumor size of 15.6 g and 4.1 g, respectively (Figures 1-4).

DISCUSSION

Differentiating giant parathyroid adenoma and parathyroid carcinoma is a diagnostic challenge. It is important to determine the risk of parathyroid carcinoma in all giant parathyroid tumour as this will determine the surgical approach of the tumour, including the resection margin and the possibility of ipsilateral thyroidectomy in the presence of nodular thyroid disease.

Giant parathyroid adenoma and parathyroid cancer share some of the common characteristics including significantly large tumour size, higher serum calcium and higher iPTH levels.[3,4]

One of the distinct properties of giant parathyroid adenoma is it can be asymptomatic[3] as presented by two cases of giant parathyroid adenoma despite high calcium and large adenoma. Thus, it can be concluded that in patients with giant parathyroid tumours with significantly high serum calcium, in the absence of symptoms, the likely diagnosis is giant parathyroid adenoma rather than parathyroid cancer.[5] However, this subject requires further evaluation.

Parathyroid carcinoma patients are frequently symptomatic with more severe symptoms.[4] Two cases with parathyroid cancer presented with severe symptoms requiring admission with significantly high calcium of 3.61 mmol/L and 4.0 mmol/L, with one of them having the largest tumour in our series weighing 38 g.

It is important to differentiate the clinical presentations of both conditions prior to surgery as fine needle aspiration is not recommended due to low discriminatory capacity and risk of dissemination.[5,6] Absence of adjacent structural invasion and distant metastases made the diagnosis even more challenging. Ultrasound plays an important part in discriminating giant parathyroid adenoma and parathyroid cancer as parathyroid cancers have lobulated and heterogenous appearance but giant adenomas have smooth borders and homogenous echogenicity.[7] The depth/width ratio is greater than 1 in 95% of parathyroid cancers and less than 1 in 94% of giant adenomas.[7]

Only histopathology examinations will confirm parathyroid carcinoma. Presence of dense fibrous bands,

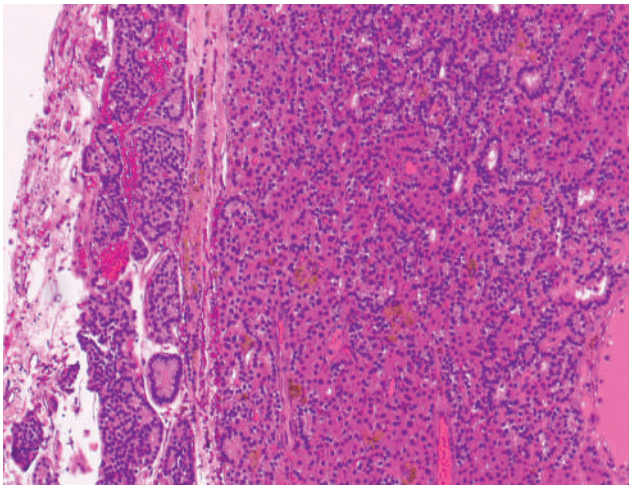

Figure 1. ***Case 21.*** Neoplastic cells arranged in compactly arranged trabecular pattern and pseudo-follicular configurations. The tumor cells are extending beyond the capsule into the attached adipose tissue (H&E, 40x).

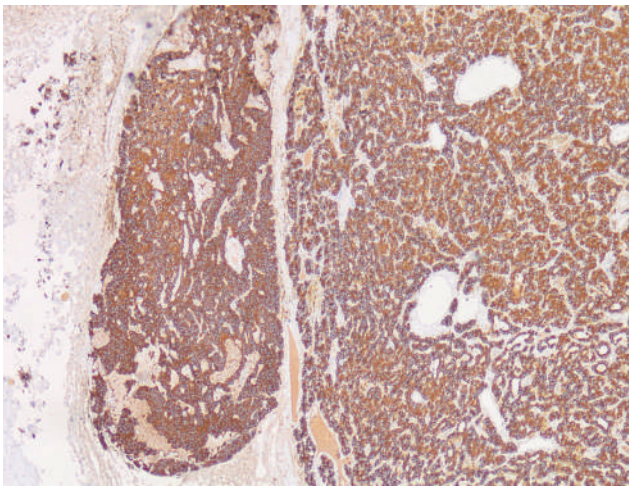

Figure 2. ***Case 21.*** Immunohistochemical studies chromogranin stain shows the tumour cells are expressing chromogranin (40x).

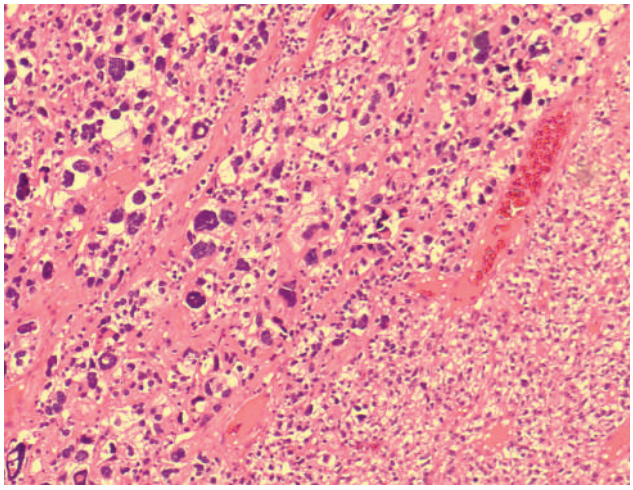

Figure 3. ***Case 15.*** Tumour cells with clear cytoplasm that are arranged in sheets and cords traversed by delicate blood vessels (right lower) and some cells with bizarre enlarged nuclei (left) (H&E, 100x).

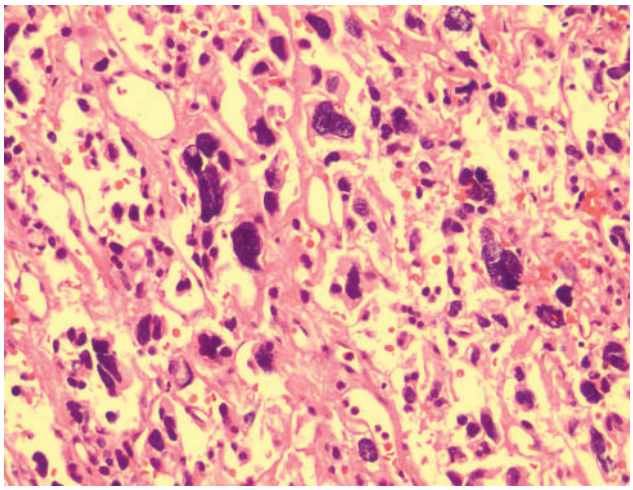

Figure 4. ***Case 15.*** Tumour cells exhibit enlarged hyperchromatic nuclei (H&E, 400x).

trabecular architecture, vascular and capsular invasion, and mitotic activity are proposed morphology criteria to identify parathyroid carcinoma although sensitivity and specificity of each isolated criterion is limited.[8] Loss of staining for parafibromin and Ki-67 of more than 5% are good indicators for parathyroid carcinoma.[9]

Another entity that is described in this case series is atypical parathyroid adenoma. Atypical parathyroid adenoma is defined by a group of intermediate form of parathyroid neoplasms of uncertain malignant potential which show some atypical histological features that represent a challenge for the differential diagnosis with parathyroid carcinomas.[10] The described atypical features include solid growth pattern, fibrous bands, and cellular atypia.[10] In our featured case, the atypical features are presence of focal capsular and perivascular invasion.

In contrast to parathyroid carcinoma, atypical parathyroid adenoma has no evidence of local invasion or metastases.[10] The outcome of patients with atypical parathyroid adenoma are less severe compared to patients with parathyroid carcinoma.[10] Reported median size and weight of atypical parathyroid adenomas by Cetani et al., are 2.5 cm (range 0.7–7.2 cm) and 4.15 g (range 0.3–101 g),[10] which is more than the weight definition of giant parathyroid adenoma (Table 2). Interestingly, O'Neal et al., reported that presence of atypical parathyroid adenoma was significantly higher than that of carcinoma among tumors weighting ≥2 g (17.5% vs 1.3%, $P < 0.05$).[11]

Study Limitations

Our case series is limited by the information gained from the electronic medical record review. Prospective case study is the optimal research methodology to further assess the differentiating criteria, the proper diagnostic approaches prior to surgery and the outcome of the approaches for better understanding of giant parathyroid adenoma and parathyroid carcinoma.

The major issue in this case series is the ethical considerations during electronic medical record review

Table 2. Clinical, radiographic and histomorphological features comparing giant parathyroid adenoma and parathyroid carcinoma

Criteria	Giant parathyroid adenoma	Parathyroid carcinoma
Clinical Features		
Symptomatology	Can be asymptomatic	Frequently symptomatic with severe symptoms
Tumour Size	Large	Large
Serum Calcium	High	High
Serum iPTH	High	High
Ultrasonography		
Features	Smooth borders and homogenous echogenicity	Lobulated and heterogenous appearance
Depth/width ratio	Less than 1	Greater than 1
Histomorphic features	Well circumscribed, with thin fibrous capsule, absent of fat cells within the mass, absent of lobular pattern[12]	Dense fibrous bands, trabecular architecture, vascular and capsular invasion and mitotic activity
Staining for parafibromin	Present	Loss
Ki-67	Less than 5%	More than 5%

and publication as there were no consent procurement from all the patients. No identifiable information was exposed. However, we had obtained approval for the publication of this case series from our local institutional review board as stated above.

CONCLUSIONS

It is important to differentiate giant parathyroid adenoma and parathyroid cancer as the clinical diagnosis will determine further surgical intervention and approach. Presence and severity of symptoms, serum calcium level, iPTH level and ultrasound features are vital aspects in discriminating giant parathyroid adenoma and parathyroid cancer prior to surgery.

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Ethical Consideration
Patient consent was not obtained during the course of electronic medical record review. The authors sought ethical clearance from the National Institute of Health, Malaysia (Ref : NIH.800-4/4/1 Jld. 82 (24) to conduct the study and publish the case series.

Statement of Authorship
All certified fulfilment of ICMJE authorship criteria.

Author Disclosure
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Padua FR, Paspe MG. Antinuclear antibody in the rheumatic and non-rheumatic diseases among Filipinos. Acta Med Philipp. 1990; 26(2):81-5.

One to Six Authors (Commentary, Online)

Krause RM. The origin of plagues: Old and new. Science. 1992;257:1073-8. PMID: 1509258. 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 Novem-ber 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.

Authors Representing a Group

Moher D, Schulz KF, Altman D; for the CONSORT Group. The CONSORT statement: Revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA. 2001;285(15):1987-91. PMID: 11308435. https://doi.org/jama.285.15.1987.

Book

Byrne, DW. Publishing your medical research paper: What they don't teach in medical school. Baltimore: Williams & Wilkins, 1998.

World Wide Web

The key and critical objectives of JAMA. http://jama.ama-assn.org/misc/aboutjama.dtl. Accessed April 4, 2007.

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Journal of the ASEAN Federation of Endocrine Societies
Unit 2005, 20th Floor, Medical Plaza Ortigas,
San Miguel Avenue, Ortigas Center,
Pasig City, Philippines 1605
Editorial Assistant: Amado O. Tandoc III, MD, FPSP
Telefax number: (+632) 8637-3162
E-mail: JAFES@asia.com; jafes.editor@gmail.com
Website: https://www.asean-endocrinejournal.org

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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.

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Review articles provide information on the "state of the art." JAFES encourages that reviews not only summarize current understanding of a particular topic but also describe significant gaps in the research, and current debates. The abstract should be from 50 to 75 words and should not be structured. A manuscript for reviews should not exceed 15 typewritten pages (including tables, figures, illustrations and references) or 4000 words.

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(Date)

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Subject: **SUBMISSION OF MANUSCRIPT FOR PUBLICATION**

We intend to publish the manuscript/, entitled "_____," under the Section *[Original Article, Review Article, Feature Article, Case Report, Case Series Interhospital Grand Rounds, Brief Communications, Letter-to-the-Editor, Special Announcements]* in the Journal of the ASEAN Federation of Endocrine Societies.

LIST OF AUTHORS

Complete Name	Position/ Designation	Institutional Affiliation	Role in writing the manuscript	Email address	ORCID iD

On behalf of all the authors, I shall act as the corresponding author with the journal from this point onward.

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JAFES Office
Unit 2005, 25th Floor, Medical Plaza Ortigas, Ortigas Center, Pasig City 1605
E-mail address: JAFES@asia.com, JAFES.editor@gmail.com
Telefax: (+632) 86373162

Assoc. Prof. Mary Ann R. Abacan, MD, MSc, FPPS
Division of Genetics, Department of Pediatrics,
College of Medicine, University of the Philippines Manila

Prof. Lorna R. Abad, MD. MSc, FPPS, FPSPME
College of Medicine, University of the Philippines Manila

Stephanie C. Andres, MD, FPCP, FPSN
Transplant Clinic, Center for Kidney Diseases,
University of Santo Tomas, España, Sampaloc, Manila

Assoc. Prof. Carl Abelardo T. Antonio, MD, MPH, IPFPH, FRSPH, AFCHSM, MIHM
Department of Health Policy and Administration,
College of Public Health,
University of the Philippines Manila

Gregory Joseph Ryan A. Ardeña, MD, FPCP, FPSEDM
Panay Health Care Hospital,
Estancia, Kalibo, Aklan, Philippines

Prof. Aye Aye Aung, MBBS, MMed Sc (Int Med), MRCP (UK), FRCP (Edin), DTM&H (London), Dr Med Sc (Gen Med), Dip Med Ed
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Assoc. Prof. Pia D. Bagamasbad, PhD
National Institutes of Molecular Biology and Biotechnology
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Mary Anne D. Chiong, MD, MSc, FPPS
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Catherine Jessica M. Lazaro, MD
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Minimally Invasive Surgery (Robotic, Laparoscopic and Endoscopic)
St. Luke's Medical Center, Quezon City, Philippines

Timothy Joseph S. Orillaza, MD, FPCR, FPSVIR, FCTMRI
The Medical City, Pasig City, Philippines

IN MEMORIAM

Dr. Jose Ma. C. Avila

JAFES EDITORIAL BOARD ADVISER
(2010-2021)

It is a sad day for the JAFES as Dr. Jose Ma. C. Avila, or Dr. Joey, joined our Creator.

Dr. Avila has served as one of the consultant advisers of JAFES from its revival in 2010 to the present, providing sound guidance to the editorial board in the operations and management of the journal, based on his years of experience as the editor-in-Chief of the Acta Medica Philippina and as one of the pioneers of the Western Pacific Region Index Medicus and Asia-Pacific Association of Medical Journal Editors.

Dr. Avila is an anatomic and clinical pathologist, an expert in childhood neoplasms and cytology, as well as a faculty of the Department of Pathology, University of the Philippines Manila College of Medicine. JAFES condoles with his family for his passing.

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