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Journal of the ASEAN Federation of Endocrine Societies

Vol. 39 No. 1 May 2024 | eISSN 2308-118x (Online)

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Generative Artificial Intelligence (AI) in Scientific Publications

Twenty-six years earlier in their famous chess rematch, an IBM Supercomputer called Deep Blue defeated then-world chess champion Garry Kasparov: it was the first-ever chess match won by a machine, a much celebrated milestone in the field of Artificial Intelligence. Just last year, the World Association of Medical Editors released the "WAME Recommendations on Chatbots and Generative Artificial Intelligence in Relation to Scholarly Publications," a recognition of not just the expanding applications of AI in scholarly publishing but more so of the accompanying emergence of concerns on authenticity and accuracy.[1] In recognition of this relevant topic, our Vice Editor in Chief, Dr. Cecile Jimeno, provided a well-attended and interesting talk during the last ASEAN Federation of Endocrine Society Convention in Thailand on the "Emerging Issues on the Use of Artificial Intelligence for Scientific Publications" (Figure 1).

In recent years, AI – particularly Generative AI – has revolutionized numerous industries, and the realm of scientific and scholarly publications is no exception. The advent of AI-driven technologies offers immense potential for enhancing research processes, from literature review to data analysis to targeted dissemination of information. However, alongside these opportunities, are rising concerns that the academic community must face to safeguard the integrity, quality, and equity of scientific research.

AI algorithms can *analyze vast datasets* more efficiently than traditional methods, uncovering patterns and insights that might otherwise remain hidden. This capability can accelerate the pace of discovery and innovation, particularly in complex fields, such as genomics, drug design and development, and translational medicine, where large-scale data analysis is often crucial.

With appropriate prompts, AI-powered tools can *assist in literature review* and even the drafting of portions of manuscripts through large language models (LLMs). There are now published articles recognizing the use of LLMs in the process of manuscript preparation.

Figure 1. JAFES Session at the 2023 AFES Convention.

https://doi.org/10.15605/jafes.039.01.01

AI-powered tools can even *assist in the peer review process*, arguably providing more objective and efficient reviews. Automated systems can screen manuscripts for plagiarism, test data integrity, and even provide preliminary assessments of methodological soundness, albeit of subject matters with relatively large body of knowledge. This capability can streamline the review process, reduce the routinary burden on human reviewers, and enhance the overall quality of published research.

AI can *facilitate more effective dissemination and accessibility of research*. Machine learning algorithms can personalize content delivery, ensuring that researchers receive the most relevant studies and updates in their field, with little need of human prompting. Natural language processing tools can aid in translating research findings into multiple languages. Although at this point as AI translators lack "content awareness" and "accuracy of context," we are amazed at how, and how fast, AI continuously learns and improves itself.

Despite these benefits, the integration of AI into scientific publication also *raises several concerns*. One of the primary issues is the potential for bias and stereotyping as could be unwittingly embedded in large sets of machine training data. Generative AI systems are only as good as the data they are trained on. If the training data reflects existing biases, the AI's outputs may perpetuate or even exacerbate these biases. This tendency can lead to skewed research findings and inequitable dissemination of knowledge, particularly disadvantaging researchers from underrepresented groups or less-resourced institutions.

Another concern is the transparency and interpretability of AI algorithms. Many AI models, particularly those based on deep learning, operate as "black boxes," making it humanly difficult to understand how they arrive at specific conclusions and specific courses of action. In the context of scientific research, where transparency of data and reproducibility of methods are not just crucial but required (i.e., Open Data), this opacity can undermine trust in AI-generated insights and recommendations.

Ethical questions also arise from the use of AI in the peer review process. While automation can enhance efficiency, there is also a risk of dehumanizing the review process and overlooking the nuanced judgment that experienced human reviewers bring to their evaluations. There is also the danger of over-reliance on AI, potentially leading to the marginalization of critical human oversight and expertise. Arguably this concern is more crucial in leading-edge research where the "context awareness" by human experts remains paramount.

However we look at it, AI has reached a tipping point and is here to stay. Over time, AI powered tools will get "smarter," more powerful, and more impactful, learning from wider use by humans. To balance the advantages of using these AI-powered tools in terms of efficiency, convenience, and speed, with the risks associated with inaccuracy, disinformation, data fabrication, stereotyping, and copyright infringement, among others, JAFES has begun revisiting and studying its policies and guidelines, from submission to peer review, from production to publication. We are actively engaging in conversations regarding Generative AI, learning with peers.

While AI has evolved and continues to evolve above and beyond that fateful chess match almost three decades ago, JAFES' commitment to publication ethics, scientific integrity, and high-quality medical publishing has grown even more.

Elizabeth Paz-Pacheco
Editor-in-Chief

Reference

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Journal of the ASEAN Federation of Endocrine Societies

ORIGINAL ARTICLE

Early Puberty Trend during the COVID-19 Pandemic in Singapore: A Retrospective Review in a Single Tertiary Centre

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Abstract

Objectives. We aimed to study the trend of referrals for precocious puberty during the COVID-19 pandemic compared to pre-COVID years, explore the differences in the demographic and clinical features, and evaluate the contributing factors.

Methodology. The cases referred for assessment of PP from 2018-2021 to our endocrine centre were grouped into pre-COVID (2018-2019) and COVID (2020-2021) years. Cases fulfilling the diagnosis of PP included the onset of thelarche <8 years in females and 4 ml testicular volume <9 years in males. The PP was further differentiated as Isolated Thelarche (IST) and Central Precocious Puberty (CPP). Early menarche was defined as menarche <10 years old.

Results. There were more referrals for PP and more diagnosed as CPP during the COVID-19 pandemic, predominantly among females. There were more endocrine tests done and more cases received treatment. None of the abnormal magnetic resonance imaging (MRI) pituitary findings required surgical intervention. The body mass index (BMI) was found to be positively associated with the risk of getting CPP with a crude-odd ratio (COR) of 1.8, P <0.001, and early menarche (COR 2.1, P <0.001).

Conclusion. We found a significant increase in the referrals of PP and diagnosis of CPP during the COVID-19 pandemic. Higher BMI was found to be associated with CPP and early menarche.

Key words: precocious puberty, early puberty, early menarche, COVID-19, obesity

INTRODUCTION

The secular trend toward earlier puberty has been observed worldwide, with many countries reporting earlier thelarche and menarche among girls. Two epidemiological studies (PROS and NHANES III) from the USA in the mid-1990s, which noted earlier sexual maturation in girls reported onset of puberty may be as early as 7.7 years in girls and as early as 7.6 years in boys.[1] The Danish study in the 1990s on the other hand, could not detect the downward secular trend in the timing of puberty.[2] Given the differences and limited epidemiological data among the countries, pubertal changes before age 8 years in girls and 9 years in boys continued to be used as the cut-off age for early or precocious puberty (PP) referrals.

The reported incidence rate of precocious puberty varies among the countries; however, most reported an increasing trend for the past 2 decades. The study of data from Danish national registries from 1998-2017 reported a sixfold increase in the incidence for girls, from 2.6 per 10,000 to 14.6 per 10,000, and a 15-fold increase for boys, from 0.1 per 10,000 to 2.1 per 10,000.[3] Similarly, the studies from Korea reported the annual prevalence of central precocious puberty (CPP) in girls and boys from 2008-2020 increased from 141.8 to 3439.9 (24.3 times) and from 2.7 to 206.5 (76.5 times) per 100,000 persons.[4] The mechanisms underlying the increasing trend in the incidence of CPP are uncertain, however, the nutritional status (overweight or obesity) has been highlighted as a major influence, especially in girls.

For the past 2 years during the COVID-19 pandemic, the incidences of PP seemed to be further accentuated and were reported in many parts of the world.[5-8] Similarly, being one of the country's main tertiary endocrine referral centres, we noticed a significant increase in the new referrals for precocious puberty and early menarche to our clinic. This prompted us to perform an audit to investigate this phenomenon.

METHODOLOGY

This is a cross-sectional study with the objective to study the change in the trend of cases referred for precocious puberty

eISSN 2308-118x (Online)
Printed in the Philippines

Received: May 25, 2023. Accepted: July 27, 2023.
Published online first: January 13, 2024.
https://doi.org/10.15605/jafes.039.01.12

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and early menarche during the COVID-19 pandemic, explore the differences in their demographic and clinical features and evaluate the possible contributing factors.

As PP is a condition with a low prevalence of about 0.02%,[9] the calculated sample size for the study was tremendously large. As such, we decided to collect all the cases that were referred during the study period. We screened through the new case endocrine clinic referral database and selected all the cases referred for PP, from the year 2018 to 2021. Missing data constituted less than 10% of the samples and hence, were excluded during the analyses.

We grouped the referrals into pre-COVID (year 2018-2019) and COVID (year 2020-2021), and retrospectively extracted the clinical data of PP cases from our electronic medical records. Cases fulfilling the diagnosis of PP were onset of puberty before 8 years in females (breast development i.e., thelarche); and less than 9 years in males (testicular enlargement i.e., increase in volume to 4 ml). Patients with thelarche of infancy presenting before 2 years old; isolated premature adrenarche; underlying structural abnormalities or oncological diseases that may directly interfere with pubertal development were excluded from the audit.

Ethics Review Board approval was exempted from the audit.

Data collection

Information collected included patient's demographic data; anthropometric measurements; Tanner staging at presentation; risk factors for precocious puberty (family history of PP, small for gestational age, prematurity, use of supplements, or other conditions such as a history of head injury/infection; twin pregnancy or adopted children); biochemical investigations including baseline luteinizing hormone (LH), follicular stimulation hormone (FSH), estradiol (E2), testosterone, and peak LH level during luteinizing hormone-releasing (LHRH) stimulation test (LH level was taken at 0, 30 and 60 minutes); radiological investigations including bone age x-ray, pelvis ultrasound, magnetic resonance imaging (MRI) of the pituitary; and treatment with gonadotrophin-releasing hormone agonist (GnRHa) injection.

Definition

The precocious puberty cases were further divided into isolated thelarche (IST) and central precocious puberty (CPP) after assessment. IST was diagnosed if the patient had thelarche with no significant bone age advancement (less than 1 year), no pubertal progression, baseline LH <0.5 and/or peak LH <5 IU/L during the LHRH stimulation test. CPP was diagnosed based on pubertal progression with significant bone age advancement (more than 1 year), LH baseline >0.5 IU/L, and/or LH peak >5 IU/L during the LHRH stimulation test. Early menarche was taken as menarche before the age of 10 years.

Statistics

Statistical analysis was performed using SPSS Statistics software version 22. Descriptive data were expressed as mean ± standard deviation (SD) and number (n) and proportion (%) for categorical variables unless otherwise stated.

Continuous variables were analysed with the independent t-test for normally distributed data and Mann Whitney Test for non-normally distributed data. The association of categorical variables was analysed with the Pearson chi-square test or Fisher's exact test, as appropriate. Simple binary logistic regression was used to determine the association between the variables. A value of P <0.05 is considered statistically significant.

RESULTS

There were 968 cases referred to New Case Endocrine Clinic from year 2018-2019 and 1069 cases from year 2020-2021. Cases referred for PP were 224 (21%) during the 2-year COVID-19 pandemic, versus 128 (13%) cases during the pre-COVID years with an increment of 8%, p <0.001. The cases that fulfilled the definition of CPP were noted to be more during COVID years with 122 (54%) cases versus 49 (38%) cases during pre-COVID years, p = 0.002. Cases of IST also increased from 23 (18%) pre-COVID to 44 (20%) cases during COVID-19 years, though the increment was not significant (Table 1).

The predominant PP referrals were females. The males comprised only 4 (6%) and 5 (3%) cases in pre-COVID and COVID years. The referrals were mostly from government hospitals and clinics (71%) compared to private centres (29%), and the proportions were very similar over the years. The majority of the patients were Chinese (74%), followed by Indian, Malay, Caucasian, and others. The age of presentation to the clinic ranges from the median age of 7.61 (interquartile range, IQR 1.64) to 7.75 (IQR 1.72) for females, and mean age 8.57 (± 2.00 standard deviation, SD) to 8.96 (± 0.89 SD) for males (Table 2).

The age of pubertal onset among female patients was quite similar during the pre-COVID years (median age 6.9, IQR 1.3) and COVID years (median age 7.0, IQR 1.0), and the majority presented with breast Tanner stage 2 (54-55%). There were more cases referred for early menarche during the COVID years, with 39 (24%) versus 15 (22%) cases, though the proportion of increment was not significant. The mean age of menarche for those with early menarche was about the same for pre-COVID and COVID years, with a mean age of 8.86 ± 0.94 SD versus 8.97 ± 0.53 SD. The onset of puberty for those cases with early menarche was median age 7.0 (IQR 0.80) during pre-COVID years, and age 7.5 (IQR 0.60) during COVID years, p <0.05 (Table 2).

The mean body mass index (BMI) standard deviation score (SDS) as well as the obese group of patients had increased

Table 1. Comparison of new case endocrine referrals pre-COVID and COVID years

Variable	Pre-COVID years, n (%)	COVID years, n (%)	*p*
New case endocrine referrals			**0.001**
a) Precocious puberty	128 (13)	224 (21) 845 (79)	
b) Other endocrine cases	840 (87)	1069 (100)	
Total	968 (100)		
Final diagnosis			**0.002**
a) Central precocious puberty (CPP)	49 (38)*	122 (54)*	
b) Isolated thelarche (IST)	23 (18)	44 (20)	
c) Not PP	56 (44)*	58 (26)*	
Total	128	224	

* = column proportions differ significantly from each other)

Table 2. Clinical characteristics of patients with precocious puberty during pre-COVID and COVID years

Variables	Pre-COVID years, n (%)	COVID years, n (%)	*p*
Gender			0.459^
a) Female	68 (94)	161 (97)	
b) Male	4 (6)	5 (3)	
Total	72 (100)	166 (100)	
Referral centre			>0.997
a) Government	51 (71)	119 (72)	
b) Private	21 (29)	47 (28)	
Ethnic			0.968^
a) Chinese	53 (74) 4 (6)	123 (74)	
b) Malay	7 (10) 4 (5) 4 (5)	11 (7)	
c) Indian		17 (10)	
d) Caucasian		6 (4)	
e) Others		9 (5)	
Age of Presentation			
a) Female	7.61 (1.64)ǂ	7.75 (1.72)ǂ	0.398ᵛ
b) Male	8.96 ± 0.89°	8.57 ± 2.00°	0.728*
Onset of puberty			
a) Female	6.9 (1.3)ǂ	7.0 (1.0)ǂ	0.074ᵛ
b) Male	8.6 (1.4)ǂ	8.5 (1.4)ǂ	0.532ᵛ
Breast Tanner Stage			0.567
2	37 (54)	89 (55)	
3	24 (36)	48 (30)	
4	5 (7)	21 (13)	
5	2 (3)	3 (2)	
Patients with menarche at presentation			0.724
a) Yes	15 (22)	39 (24)	
b) No	53 (78)	122 (76)	
Age of menarche	8.86 ± 0.94°	8.97 ± 0.53°	0.539*
Onset of puberty in early menarche	7.00 (0.80)ǂ	7.50 (0.60)ǂ	0.035ᵛ
BMI SDS			
a) Female	0.26 ± 1.24°	0.43 ± 1.10°	0.307*
b) Male	0.62 ± 0.76ǂ	1.87 ± 0.33°	0.012*
Height SDS			
a) Female	0.87 ± 0.96°	0.93 ± 1.04°	0.692*
b) Male	1.04 ± 1.66°	2.19 ± 1.50°	0.312*
Weight SDS			
a) Female	0.65 ± 1.08°	0.79 ± 1.02°	0.374*
b) Male	0.93 ± 0.85°	2.25 ± 0.65°	0.033*
Weight category			0.383
a) Obese	9 (12)	33 (20)	
b) Overweight	13 (18)	29 (17)	
c) Not overweight/obese	50 (70)	104 (63)	
Overweight/Obese			
a) Female	21 (31)	57 (35)	0.509
b) Male	1 (20)	5 (100)	0.048^
Family history PP			0.861
a) Yes	9 (12)	22 (13)	
b) No	63 (88)	143 (87)	
Risk factors of PP			0.059^
a) SGA	2 (3)	11 (7)	
b) Prematurity	5 (7)	13 (8)	
c) Supplements	2 (3)	12 (7) 9 (5)	
e) Others	3 (4)	121 (73)	
f) No	60 (83)		
Received treatment		45 (27)	0.048
a) Yes	11 (15)	121 (73)	
b) No	61 (85)		

°mean ± SD; ǂmedian (interquartile range); *Independent-Samples T-test; ^Fisher's Exact test; ᵛMann-Whitney test

Table 3. Association of overweight/obesity with diagnosis of CPP, IST and early menarche

Factors	Overweight/Obese, n (%)	OR (95% CI)	*p*-value
CPP			
No	16 (19)	Ref	- 0.023
Yes	68 (81)	2.1 (1.1 - 4.0)	
IST			
No	68 (81)	Ref	- 0.023
Yes	16 (19)	2.1 (1.1 - 4.0)	
Early Menarche			
No	44 (56)	Ref	-
Yes	34 (44)	3.4 (1.8, 6.2)	<0.001

Ref = reference; OR = odds ratio; CI = confidence interval

Table 4. Diagnostic features of patients with precocious puberty during pre-COVID and COVID years

Variables	Pre-COVID years, n (%)	COVID years, n (%)	*p*
Baseline blood investigations done			0.285
a) Yes	39 (54)	101 (62)	
b) None	33 (46)	63 (38)	
LHRH test done			**0.043**
a) Yes	8 (11)	37 (22)	
b) No	64 (89)	129 (78)	
Done LHRH test			
a) Female	7 (88)	37 (100)	0.278
b) Male	1 (12)	0	0.382^
LHRH test results			0.133^
a) Positive	7 (88)	22 (60)	
b) Negative	1 (12)	15 (40)	
USS pelvis done			0.846
a) Yes	24 (35)	59 (37)	
b) None	44 (65)	102 (63)	
Ovary volume (ml)			
a) IST	0.55 ± 0.05°	1.37 ± 0.54°	0.097*
b) CPP	3.00 ± 2.36°	2.84 ± 1.09°	**0.001***
MRI pit			0.064
a) Done	20 (28)	67 (40)	
b) Not Done	52 (72)	99 (60)	
MRI pit			0.260
a) Normal	12 (60)	49 (73)	
b) Abnormal	8 (40)	18 (27)	
Abnormal MRI pit			
a) Female	7 (44)	17 (27)	0.207
b) Male	1 (25)	1 (20)	0.722^

°mean ± SD; *Independent-Samples T-test; ^Fisher's Exact test; ᵛMann-Whitney test

from pre-COVID to during the COVID years, though the majority of referred cases were not overweight or obese. The proportion of overweight/obese patients for both genders has increased over the years, but we noticed a significant increment among the male patients during the COVID year, with an increase in both the weight SDS and BMI SDS (Table 2). We found that the obese/overweight patients were significantly higher among those diagnosed with CPP as compared to IST, with 68 (39.8%) versus 16 (23.9%), p <0.05; and was found to be positively associated with the risk of getting CPP with odd ratio (OR) 2.1, P = 0.023; as well as early menarche (OR 3.4, P <0.001) (Table 3).

The majority of the patients did not have any obvious risk factors for PP. About 12-13% of them had a family history of PP, and 7-8% were born prematurely. More patients in COVID years who were born small for gestational age presented with PP 11 (7%) versus 2 (3%) and taking supplements either oral or topical with 12 (7%) versus 2 (3%) cases in pre-COVID years (Table 2). None of the risk factors were found to be significantly associated with PP or CPP.

More baseline hormonal tests were done during the COVID years though the differences were not statistically significant. A more significant number of LHRH stimulation tests was done during the COVID years, with 37 (22%) vs 8 (11%) cases in pre-COVID years, and more proportion were carried out in female patients (Table 4). There was a significantly higher proportion of patients who received GnRHa treatment during COVID years with 45 (27%) versus 11 cases (15%), p <0.05 (Figure 1).

More ultrasound pelvis and MRI pituitary imaging were done during the COVID years, though these were not statistically significant. Ovarian volume was noted to be smaller in the IST group as compared to the CPP group during the pre-COVID year but with a slight difference during the COVID years. Eight (40%) and 18 (27%) of the MRI pituitary done during pre-COVID and COVID years were found to be abnormal, with female predominance (Table 4). However, none of them revealed pathological brain lesions that required surgical intervention. There were 7 cases with macroadenoma or microadenoma; five with Rathke's cyst; two with hypothalamic hamartoma; five with slight pituitary enlargement and others with

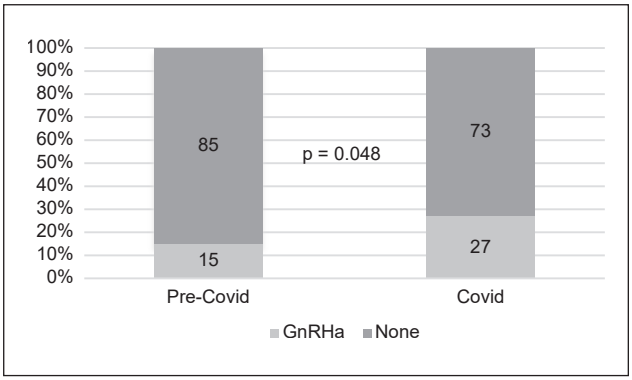

Figure 1. Percentage of patients started on GnRHa during pre-COVID and COVID years.

incidental findings such as pineal cyst, arachnoid cyst and non-specific lesions in the brain.

DISCUSSION

The COVID-19 pandemic has changed the world drastically, not only in terms of COVID infection-related mortality and morbidity rate but also in the lifestyle modification of human beings. The strict lockdown and school closure had caused the children and adolescents to mostly stay indoors, resulting in reduced physical and social interaction, and increased use of electronic devices, which resulted in an overly sedentary lifestyle. People were stressed and confined at home and ate more frequently. All these changes have led to an increasing rate of obesity in many countries, especially among children who were mostly kept protected and confined at home.[10,11] Many endocrine centres around the world have reported an increased incidence of early puberty, faster pubertal progression as well as early onset of menarche during the COVID pandemic, with many publications from Italy, which was one of the hardest-hit countries by COVID-19 infection during the early pandemic.[5,12-15]

The literature and studies have tried to link the causal associations between increased incidence of early puberty with a sedentary lifestyle, obesity, overnutrition, exposure to endocrine-disrupting agents, as well as the use of electronic devices.[5,13] Chen, Yao et al., published a study that suggested an increased incidence of precocious puberty during the COVID-19 pandemic among Shanghai school-aged girls may be associated with decreased serum concentrations of MKRN3 and ghrelin.[6] Kisspeptin and leptin promote the pulsatile GnRH secretion, whereas MKRN and ghrelin suppress it. Rapid weight gain leads to increased concentrations of leptin but decreased concentrations of ghrelin. However, the actual cause remains unknown.

From our study, we found an increase in the number of patients referred and diagnosed with PP during the COVID-19 pandemic years, with more diagnosed as CPP with progressing puberty as compared to IST. There were also more patients referred for early menarche, though the increment was not significant. We noticed that the reported onset of puberty for those with early menarche was significantly later during the COVID years. This may suggest that puberty progressed faster for the PP cases during the COVID years, as reported by some published papers.[12] However, it is difficult to determine the exact onset of puberty for most cases of PP and early menarche, as most parents and patients did not notice the early signs of puberty. This is especially difficult in males where the first sign of puberty is testicular size increment.[8]

In our study, we noticed there were more endocrine stimulation tests (LHRH) performed and more patients treated with GnRHa during the COVID years. LHRH stimulation test usually was performed if the baseline investigations and clinical features were not conclusive of central precocious puberty, typically during the early puberty stages. This may suggest that more cases with early stages of puberty were referred during the COVID years, possibly due to increased awareness of CPP among clinicians and parents. This could be the influence of social media and more exposure to online educational talk during the pandemic years. Similar reasons could explain why more treatment with GnRHa was received during the COVID years, with earlier diagnosis and higher acceptability of the treatment by clinicians and parents.

Our study found higher BMI is associated with a higher risk of CPP and early menarche, which is consistent with some studies.[7,12] We did not notice an increase in cases of PP in males during the COVID years, similar to other reports.[8] However, we noticed the proportion of overweight/obese male PP patients had significantly increased during the COVID years. This is likely related to the increased incidence of obesity during the COVID-19 pandemic.[10,11] Apart from the risk factor of overweight/obese; we did not find other significant risk factors that were associated with PP or CPP.

We found more pituitary MRIs were done during the COVID years with less proportion of abnormalities detected, though the increment was not significant. The cases of abnormal MRI imaging detected were mostly benign and stable lesions not requiring surgical intervention, as reported by other studies.[5] However, it is difficult to make many conclusions from these data, especially for male patients, due to the small sample size. Patients with significant pituitary lesions usually were referred to with more clinical features than just isolated PP. Besides, we excluded cases with under-lying structural abnormalities or oncological diseases that may directly interfere with pubertal development.

The increase in the incidence of precocious puberty with faster progression observed during this COVID-19 pandemic is a worrisome phenomenon. However, it also opened a new window of opportunity for clinicians and researchers to further study the science behind the

accelerated pubertal onset and its impact on children and adolescents.

Our study is limited by its retrospective design done in a single centre which reduces the sample size and ability to objectively study the association of various risk factors associated with PP. A prospective multi-centre study in the future should be the way forward.

CONCLUSION

We found an increase in the number of female patients referred and diagnosed with PP during the COVID pandemic years, with more endocrine stimulation tests (LHRH) done, more diagnosis of CPP and administration of GnRHa treatment. More male PP patients were noted to be overweight or obese. Higher BMI was found to be associated with a higher risk of CPP and early menarche. Apart from the risk factor of overweight/obese; we did not find other significant risk factors associated with PP; and there were no other significant differences in the demographic and clinical features of the referred PP cases during the pre-COVID and COVID years.

More causal reasons and environmental factors must have contributed to the increase in PP, as most of the referred PP female cases were not overweight or obese. It would be important to monitor the subsequent trend of puberty in children after the COVID-19 pandemic and consider a more extensive prospective study involving multiple centres.

Acknowledgments
We acknowledge our statisticians Rehena Ganguly from Singhealth and Wilson Low Cong from KK Women's and Children's Hospital for their contribution.

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

CRediT Author Statement
AL: Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **RFV:** Conceptualization, Methodology, Resources, Writing – review and editing, Supervision

Author Disclosure
Both authors declared no conflict of interest.

Funding Source
None.

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Journal of the ASEAN Federation of Endocrine Societies

ORIGINAL ARTICLE

Endocrine Disorders in Childhood Brain Tumour Survivors: A Single-Centre Study

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Abstract

Objective. The study aims to determine the prevalence and risk factors for endocrine disorders in childhood brain tumour survivors.

Methodology. Included in the study were 124 childhood brain tumour survivors aged 18 years old or younger with either stable disease or in remission, and had survived for at least 2 years after diagnosis. Demographic data (age at diagnosis, gender, ethnicity, socioeconomic status), clinical clues for endocrine disorders, anthropometrics (weight, height, midparental height), pubertal staging, tumour-related characteristics, treatment modalities and endocrine laboratory measurements at diagnosis and during follow up were obtained. Logistic regression was applied to evaluate risk factors for endocrine disorders in childhood brain tumour survivors.

Results. The prevalence of endocrine disorders in childhood brain tumour survivors was 62.1%. The risk factors were high BMI [adjusted odds ratio (OR) 1.29, 95% CI: 1.12 to 1.5], high-risk site [adjusted odds ratio (OR) 7.15, 95% CI: 1.41 to 36.3] and chemotherapy [adjusted odds ratio (OR) 0.18, 95% CI: 0.05 to 0.62].

Conclusion. The prevalence of endocrine disorders in childhood brain tumour survivors in our centre was 62.1%. The significant risk factors were high BMI, tumour location (suprasellar and intrasellar) and chemotherapy.

Key words: endocrine disorder, childhood brain tumor survivors, risk factors

INTRODUCTION

Brain tumours are the commonest type of paediatric solid organ tumours, and it is the second most common childhood malignancy after leukaemia which contributes to 21% of all paediatric malignancies.[1] Its prevalence varies among different countries, with the highest rates reported in the United States.[2] The average annual incidence of primary CNS tumours for children and adolescents ≤19 years old in the United States from 2011 to 2015 was 5.95 cases per 100,000 population.[2] Approximately 60 percent of cases were malignant and 40 percent were non-malignant. According to the Malaysian National Cancer Registry Report 2007–2011, the national incidence of childhood brain and central nervous system (CNS) tumours was 2 per 100,000 children.[3] There were lower incidence rates that have been reported in other parts of the world, such as Japan (estimated incidence 3.61 per 100,000 children) and Italy (3.46 per 100,000 children).[4]

The mortality of childhood brain tumour exceeds the mortality rate of acute lymphoblastic leukaemia, making it the leading cause of childhood cancer-related deaths.[4] Their prognosis and survival rates depend on multiple factors including the histological type, size and location of the tumour. The survival outcomes in childhood brain tumours have improved significantly due to the advances in diagnosis and treatment, as well as the understanding of the disease aetiology.[5]

With improved survival rate, there has been a rising concern regarding the late sequelae of childhood brain tumour survivors, particularly associated with the use of craniospinal radiation therapy (RT) in young children. Their long-term complications such as neurological impairments, cognitive dysfunction, growth and endocrine disturbances have increased. Many survivors will face numerous lifelong health-related challenges after curative treatment of a childhood brain tumour.[5]

eISSN 2308-118x (Online)
Printed in the Philippines

Received: April 10, 2023. Accepted: July 5, 2023.
Published online first: December 22, 2023.
https://doi.org/10.15605/jafes.039.01.05

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Some of the potential risks for developing endocrine complications are age at cancer diagnosis, tumour histology, location and radiation exposure. Cranial radiotherapy is the main cause of hypothalamic and pituitary injury resulting in hormonal deficiency in children with brain tumours.[6] A recent analysis of the St. Jude Lifetime Cohort revealed that there was at least one anterior pituitary hormone deficiency in 51.4% of childhood cancer survivors who received cranial radiotherapy.[7]

Our findings would contribute to the paucity of data regarding the prevalence and risk factors of brain tumour survivors not only locally but also worldwide, and this clinical information would aid in improving the management of patients who are at risk for endocrine disorders.

METHODOLOGY

Study design and setting

We conducted a cross-sectional study at the Hospital Universiti Sains Malaysia (HUSM). The subjects were patients with brain tumours who were diagnosed from January 2002 till December 2017. It was approved by the Human Research Ethics Committee USM (reference: USM/JEPeM/21010039).

Subjects and procedures

We included all patients aged 18 years old and who survived for 2 years or more after diagnosis; had stable residual disease or no evidence of disease progression at the time of follow-up. We excluded patients who were syndromic, patients with incomplete data, had pre-existing endocrine disorders which were diagnosed before the tumour and congenital causes of endocrine disorders such as septo-optic dysplasia.

Records were traced from the medical record unit. Demographic data (age at diagnosis, gender, ethnicity and socioeconomic status), tumour-related characteristics and treatment modalities were extracted from medical records. We also collected clinical data (clues for endocrine disorders), anthropometrics (weight, height, midparental height), pubertal stage according to Tanner staging, endocrine laboratory measurements at diagnosis and during follow-up. All data collected were recorded in the data collection sheet.

Anthropometric measurements were plotted using World Health Organization growth chart for children less than 5 years old and National Centre for Health Statistics chart for children who are more than 5 years old. We included weight and height reviewed by the managing team before starting any treatment such as growth hormone in our analysis. BMI was calculated using the latest weight and height. The tumour location was classified as high-risk and low-risk. We analysed endocrine abnormalities detected even years after the diagnosis of brain tumour and had persisted beyond 2 years.

Sample size estimation

The sample size requirement for the estimation of the prevalence of endocrine disorders was determined using the sample size formula for estimation of proportion, $n = (Z_\alpha/\Delta)^2 P(1-p)$, where P is the observed prevalence reported from the previous study, Δ is the margin of error and Z_α is the Z-value corresponding to the level of confidence. For an estimation with a 95% confidence level, 10% margin of error and prevalence of 61%, as Ng et al., reported, the required sample size is 92 patients.

The sample size required to determine factors associated with endocrine disorders was calculated using the calculation for logistic regression analysis in G*power software version 3.1.9.7 (Test family: Z-test; Statistical test: Logistic regression). Sex was a significant predictor of endocrine disorders from a previous study, with females having a higher risk of developing endocrine disorders. It has been reported that the percentage of male patients with endocrine disorders was 36.5%. To achieve 80% study power with type I error of 5% (two-tailed), odds ratio (OR) of 3, R^2 contributed by other factors of 0.02, and equal ratio between sex, the required sample size was 113 patients. Considering a 10% possibility of missing data, the corrected sample size was 126 patients.

Statistical analysis

Data analysis was done using Statistical Package for Social Science (SPSS) IBM version 26.0. All data were checked for their distribution with histogram and probability plots. Numerical variables with normal distribution were presented as mean and standard deviation (SD). Non-normally distributed numerical variables were presented as median and interquartile range (IQR). Categorical data were presented as frequency (percentage).

Logistic regression analysis was used to determine factors associated with endocrine disorders in brain tumour survivors. Simple logistic regression analysis was used to identify factors to be included in the multiple regression analysis. Cut-off was set at $p<0.25$ in determining variables to be included in the final model. For multiple logistic regression we used backward stepwise regression. The selection starts with all independent variables in the model and then remove those with the largest p-value, one at a time. Criteria to retain the variable was set at $p<0.05$. All the assumptions of the test were examined. The fitness of the model was assessed using Hosmer-Lemeshow test. Outlier and influential observations were examined using Cook's influential statistics, while linearity was examined using the Box-Tidwell procedure. The area under the curve was 0.812. The factors that remained in the final model were presented using a table with its corresponding adjusted odds ratio, 95% CI, and p-value.

RESULTS

Demographic, clinical and hormonal characteristics are summarized in Table 1. The majority were female at 54% compared to male at 46%. The predominant race was Malay (96%). The majority of the subjects were in the low socioeconomic group (83.9%). The subject's mean age at diagnosis of brain tumour was 8.6 years (SD 8). The median body weight of the patients was 32 kg (IQR 19-45) and their median height was 135 cm (IQR 112-152). The median BMI was 17 kg/m^2 (IQR 11-41). For age distribution and BMI percentile, (refer to Appendix), 66.7% had 95th BMI percentile for the age range 12-18 years old, 33.3% had >97th BMI percentile for age 6-12 years old, while 22.2% of age 12-18 years old had >97th BMI percentile. There were 20.2% subjects with tumours at high-risk sites. Most of the subjects underwent intracranial surgery (91.9%). Other treatments received by patients were radiotherapy 38.7%, chemotherapy 16.9% and chemoradiation 11.3%.

There were 62.1% patients with endocrine disorders (Table 1). The age distribution of involved patients was highest at age 6-12 years old (45.5%); followed by those aged 2-5-years-old (31.2%); 13-18 years old (20.8%) and <2 years (2.6%). The proportion of hypopituitarism was 6.5%, panhypopituitarism was 18.5% while isolated central hormonal deficiency was 4.8%. The most common hormonal deficiency was hypothyroidism (26.6%) followed by growth hormone deficiency (GHD) (21%), gonadotropin deficiency (21%) and ACTH deficiency (20%). Most of the specific endocrine disorders (refer to Appendix) occurred in those aged 6-12 years old, namely hypothyroidism (51.5%), growth hormone deficiency (57.7%), ACTH deficiency (64.4%), gonadotropin deficiency (57.7%), delayed puberty (56.1%), short stature (54.4%), obesity with metabolic syndrome (38.1%0, SIADH (75%) and cranial diabetes insipidus (55.6%). For cerebral salt wasting, it was predominantly seen in those 2-5 years old (43.8%).

From Table 2, the commonest clinical manifestations of endocrine disorders were short stature (46%) and obesity with metabolic syndrome (33.9%). Other clinical manifestations were delayed puberty (33%), oliguria secondary to syndrome of inappropriate secretion of anti-diuretic hormone (SIADH), (3%), polyuria secondary to cerebral salt wasting (CSW), (12.9%), and polyuria secondary to cranial diabetes insipidus (DI) (14.5%).

Figure 1 summarizes the tumour histology of our subjects. The commonest tumour was medulloblastoma (26.6%), followed by astrocytoma (25%) and glioblastoma (11.3%). The proportion of ependymoma was 7% while the proportion of pituitary adenoma and primitive neuroectodermal tumour (PNET) were both 6%. Regarding medulloblastoma and age category (refer to Appendix), 63.6% affected those 6 to 12 years old, 21.2% affected those 2 to 5 years old, 9.1% among 12 to18 years old and 6.1% in those less than 2 years old.

From simple logistic regression analysis, BMI, weight, household income, site of brain tumour, hydrocephalus at diagnosis, chemotherapy and chemoradiation were

Table 1. Demographic, clinical and hormonal characteristics

Variables	n (%)
Age at diagnosis (years)	8.6 (5.0)[c]
Weight (kg)	32.0 (19-45)[a]
Height (cm)	135.0 (112-152)[a]
BMI (kg/m^2)	17.0 (11-41)[a]
Sex	
Boy	57 (46)[b]
Girl	67 (54)[b]
Race	
Malay	119 (96)[b]
Others	5 (4)[b]
Income	
B40	104 (83.9)[b]
Non-B40	20 (16.1)[b]
High-risk site	25 (20.2)[b]
Surgery	114 (91.9)[b]
Radiotherapy	48 (38.7)[b]
Chemotherapy	21 (16.9)[b]
Chemoradiation	14 (11.3)[b]
Endocrine disorders	77 (62.1)[b]
Hypopituitarism	8 (6.5)[b]
Panhypopituitarism	23 (18.5)[b]
Isolated hormone deficiency	6 (4.8)[b]
Growth hormone deficiency	26 (21)[b]
ACTH deficiency	25(20.2)[b]
Gonadotropin deficiency	26 (21)[b]
Hypothyroidism	33 (26.6)[b]

[a] Height, Weight, Body mass index (BMI) are not-normally distributed and presented as median [interquartile range (IQR)]
[b] Frequency and percentages for categorical variables
[c] Age is normally distributed and presented as mean [standard deviation (SD)]
B40: total household income < RM4850, Non-B40: total household income other than B40, High-risk site: intrasellar and suprasellar region, Low-risk site: other locations, ACTH: Adrenocorticotropic hormone.

Table 2. Clinical manifestation of endocrine disorders

Variables	n (%)
Short stature	57 (46)[b]
Polyuria secondary to CSW	16 (12.9)[b]
Obesity with metabolic syndrome	42 (33.9)[b]
Delayed puberty	41 (33.1) [b]
Oliguria secondary to SIADH	4 (3.2)[b]
Polyuria secondary to Cranial DI	18 (14.5)[b]

CSW: Cerebral salt wasting, SIADH: Syndrome of inappropriate secretion of antidiuretic hormones, DI: Diabetes Insipidus

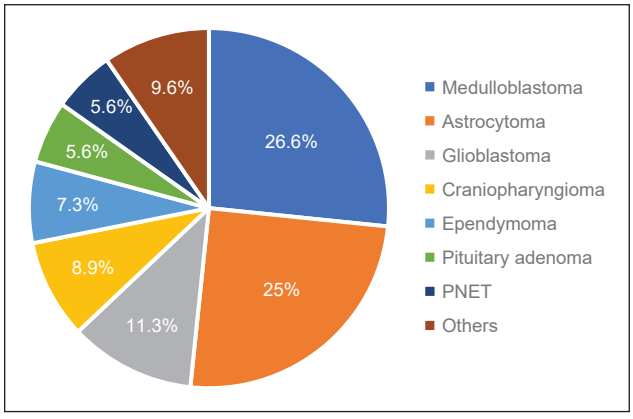

Figure 1. Histology.

[a] Others (%): oligodendroglioma: (1.6%), meningioma: (1.6%), Schwannoma: (0.8%), germ cell tumour: (2.4%), germinoma:(3.2%)

Table 3. Factors associated with endocrine disorders in childhood brain tumour survivors

Variable		Crude OR[a] (95% CI)	Adjusted OR[b] (95% CI)	Wald stat[b] (odd)	P value[b]
Body Mass Index (BMI)		1.28 (1.13, 1.45)	1.29 (1.12, 1.50)	12.06 (1)	0.001
High risk site	Yes	9.58 (2.14,42.87)	7.15 (1.41, 36.25)	5.64 (1)	0.018
	No	1.00	1.00		
Chemotherapy	Yes	1.00	1.00	7.27(1)	0.007
	No	0.33 (0.10, 1.05)	0.18 (0.05, 0.62)		

[a] Simple logistic regression
[b] Multiple logistic regression
OR: Odd ratio, CI: Confidence interval
The model reasonably fits well. Model assumptions are met. There are no interactions and multicollinearity problems.

important variables with $p<0.25$. Statistically significant variables from multiple logistic regression analysis (Table 3) were high BMI (adjusted OR 1.29, 95% *CI*: 1.12 to 1.5, p =0.001), high-risk site (adjusted OR 7.15, 95% *CI*: 1.41 to 36.3, p=0.018) and no chemotherapy (adjusted OR 0.18, 95% *CI*: 0.05 to 0.62, p=0.007). Brain tumour survivors with high BMI have an increased odds of having endocrine disorders by 1.2 times. Those survivors with brain tumours at high-risk sites have increased odds of having endocrine disorders by 7.2 times than those at lower risk sites. Brain tumour survivors that received no chemotherapy have decreased odds of having endocrine disorders by 83% than those who received chemotherapy.

DISCUSSION

The prevalence of endocrine disorders among childhood brain tumour survivors (CBTS) in our centre was 62% which was almost similar to a local study conducted in Klang valley by Ng et al, 61%.[8] Our prevalence was higher compared to other studies done in South Korea (37.1%), Netherlands (22.1%) and United States (49%).[2,9,10] Studies by Clement et al., in the Netherlands excluded patients with craniopharyngioma or a pituitary gland tumour while our study not only included all childhood brain tumour survivors, but also all types of endocrine complications such as obesity, growth, pubertal disorders and water/salt disturbances.[11] A study by Heo et al., only included patients based on insurance claim with limited coding for endocrine disorders and therefore explains the lower prevalence in South Korea.[10] The lower prevalence in other centres was also explained by a lack of standard guidelines for surveillance of endocrine disorders, inconsistent follow up, delayed referral to paediatric endocrinologist and lack of awareness among doctors in managing CBTS.[12,13]

Most endocrine disorders occur 2 to 3 years after diagnosis of the brain tumour.[14] Based on the age categories for our centre majority affected those 6 to 12 years old, followed by those 2 to 5 years old (31.2%), 13 to 18 years old (20.8%), and lastly, those less than 2 years old (2.6%). The median age of our patients diagnosed to have primary brain tumour with or without endocrine disorders was 8.6 years compared to Ng et al., which was 5.6 years and Clement et al., at 8.5 years old.[10,11] We were unable to compare the age categories of patients with endocrine disorders with other centres due to lack of data.

The most frequent type of brain tumour confirmed by histopathological examination in our centre was medulloblastoma (26%), which is similar to many other publications, accounting for 25 to 30% of childhood brain tumours.[15] For the age distribution in our centre, 63.6% affected those aged 6 to 12 years old and tended to affect the posterior fossa. Most present with signs of increased intracranial pressure, altered mental state, cerebellar ataxia and focal neurological deficits.[16] Other types of brain tumour based on biopsy findings varied between centres and this may reflect different studied populations, ethnicities and possibly genetic heterogenicity.

Regarding specific pattern of hormonal deficiency in our centre, hypothyroidism was the commonest (26.6%) followed by GHD (21%), gonadotropin deficiency (21%) and ACTH deficiency (20%). Ng et al., reported ACTH deficiency was the most prevalent (37%) followed by hypothyroidism (35%) and CDI (21%).[8] In other parts of the world, the commonest hormonal deficiency was GHD that ranged from 12 to 58% and this pattern was seen more consistently compared to other hormonal deficiencies,[17-19]

Short stature (46%) was the most common clinical presentation seen in our centre and the causes could be multifactorial, such as specific hormonal deficiency secondary to GHD, hypothyroidism, ACTH deficiency, poor nutrition, familial short stature or constitutional delay in growth and puberty (CDGP).[19] This finding was also reported by Gurney et al, and Pasqualini et al.[20,21] Despite it being the most common manifestation, not all the CBTS were referred to endocrine unit for screening of the specific hormonal deficiency which might be due to poor awareness among the primary doctors.

The majority of the salt and water disturbances occur in the immediate post-operative period such as cranial diabetes insipidus (CDI), cerebral salt wasting (CSW) and SIADH.[22] Most are transient and resolved around 4 to 6 weeks post-operation.[23] However, some of the water/salt disturbances persist years after the insult particularly those with residual tumour, and in our centre, persistent CSW and CDI contributed about 12.9% and 14.5%, respectively.

There are many risk factors for the development of endocrine complications in CBTS. One of the potential risks is the site of brain tumour. The high-risk sites are the hypothalamus and pituitary regions.[19] Any insults due to

surgery, radiotherapy and the primary tumour itself may cause damage to the hypothalamus/pituitary and result in a hormonal deficiency. The radiation dose of at least 18 Gy from TBI is associated with GHD as somatotroph cells are very sensitive to ionising radiation. A dose of radiation above 50 Gy may result in multiple pituitary hormonal deficiency or hypopituitarism.[6] Our finding was consistent with Clement et.al., and Lawson et. al., but the tumour location was not found to be a significant factor in a study by Ng et al.

The hypothalamic form of obesity is an important endocrine complication commonly encountered in CBTS.[24] Hypothalamic arcuate and paraventricular nuclei are the appetite centres that regulate satiety and hunger, and they form a complex pathway for food intake and energy expenditure.[25] The main mechanism of hypothalamic obesity is damage to hypothalamic nuclei secondary to surgery, radiotherapy or primary brain tumour which results in interference of afferent sensory input and energy regulation leading to hypothalamic hyperphagia and obesity.[24] Other associations were excessive consumption of unhealthy food and sedentary lifestyle related to neurological/motor dysfunction a few years after surgery/radiotherapy.[24] Our finding was consistent to Lutsig et al., and Cooksey et al., that concluded obesity was a known complication in CBTS.[26,27]

Chemotherapy is another factor associated with endocrine complications such as delayed puberty and infertility.[28] Most often it is associated with the use of cyclophosphamide, ifosfamide, and busulfan.[28]

We found that 33% of CBTS had delayed puberty. The prevalence of pubertal delay varies between studies with a range of 4 to 11%.[8,11] It is most often underrepresented due to missing pubertal assessment or short duration of follow up because the median age of diagnosis was at prepubertal age. Alkylating agents cause gonadal toxicity and results in a hypergonadotropic form of hypogonadism or primary hypogonadism.[29] Affected subjects would have absence or poor progression of puberty and may be infertile later on. Other factors that may contribute to delayed puberty and infertility are radiotherapy, poor nutrition and abnormal body composition.[30]

Limitations of the study

Our study had a few limitations as it was retrospective in nature. We managed to trace 290 records using keywords of brain tumour. One hundred sixty-six were excluded since 98 of them were adults, 50 already died and 24 had missing records. A few years before the endocrine service was started, hormonal levels were not regularly checked during follow-up which may be the reason for missed diagnosis of specific endocrine disorders. There was no screening for osteoporosis in the studied subjects which is a known complication among CBTS. Subjects selected were limited to our hospital, which might not truly represent the childhood brain tumour survivors in our state. Lastly, all CBTS need a longer follow-up as endocrine complications may occur as long as 20 years following initial diagnosis of brain tumour.

CONCLUSION

The prevalence of endocrine disorders in childhood brain tumour survivors in our centre was 62%. The risk factors were high BMI, tumour location (suprasellar and intrasellar) and chemotherapy.

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

Credit Author Statement
NWR: Conceptualization, Methodology, Software, Validation, Investigation, Resources, Data Curation, Writing - original draft preparation, Visualization, Project administration, Finding acquisition; **SH:** Software, Validation, Formal analysis, Resources, Data Curation, Writing - review and editing, Visualization, Supervision; **NM:** Validation, Supervision; **NMY:** Software, Validation, Formal analysis, Supervision

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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Journal of the ASEAN Federation of Endocrine Societies

ORIGINAL ARTICLE

Validation of the Gestational Diabetes Mellitus Knowledge Questionnaire (GDMKQ) among Filipino Patients in a Tertiary Medical Center

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Abstract

Objectives. Gestational diabetes mellitus (GDM) is a common pregnancy complication with adverse fetal and maternal outcomes. Currently, there are only a few validated tools available that address knowledge in GDM. Recognition of the inconsistencies will provide an effective learning program to achieve optimal results. This study aimed at validating the "Gestational Diabetes Mellitus Knowledge Questionnaire" (GDMKQ).

Methodology. A cross-sectional validation study on GDMKQ among 51 GDM patients aged at least 18 years was conducted in the outpatient clinics of a tertiary hospital. Excluded were those with pre-existing diabetes. The questionnaire was submitted for peer review for translation to Filipino and back-translation. Concurrent validity, internal consistency and test-retest reliability of the questionnaire were undertaken as part of the validation process. Descriptive analysis was used for data elaboration by using SPSS v23.

Results. The Filipino version of GDMKQ demonstrated sensible content and face validity. As measured, respondents obtained higher total and domain scores with better knowledge levels of GDM compared to its English version. Overall adequate knowledge was observed among those married and college subgroups as compared to single women and those with secondary levels of education. The reliability of the questionnaire was calculated at 0.632 using the Kuder-Richardson 20. The test-retest scores using the Filipino-translated questionnaire have a Pearson correlation coefficient of 0.853 with moderate to good level of agreement with each other, and Cohen's kappa of 0.564 with an intra-class correlation coefficient of 0.828.

Conclusion. The Filipino-translated version of GDMKQ is a valid screening tool that assesses a patient's knowledge on gestational diabetes. Identifying the level of their understanding will enable clinicians to develop an individualized, effective learning program to improve pregnancy outcomes.

Key words: gestational diabetes, knowledge, questionnaire, validation

INTRODUCTION

Pregnancy is associated with hyperinsulinemia and insulin resistance which may cause some patients to develop diabetes mellitus (DM).[1] DM is responsible for 2 to 5% of pregnancy complications, and 90% of these cases are caused by gestational diabetes mellitus (GDM).[2,3] According to the International Association of Diabetes and Pregnancy Study Groups (IADPSG), GDM is defined as any degree of glucose or carbohydrate intolerance that is first diagnosed during pregnancy.[1-6] It occurs in 0.6 to 20% of the pregnant population worldwide.[1,5] The prevalence differs across geographical settings but was found to be highest among Asians. In the Philippines, it affects 14% of pregnancies as reported by the Asian Federation of Endocrine Societies Study Group on Diabetes in Pregnancy (ASGODIP).[7] Predisposing factors include ethnicity (Asian, African, Hispanic, Native American and Pacific Island descent), overweight pre-pregnant body mass index (BMI), age of mother (more than 25 to 35 years old), family history of DM or previous history of GDM, sleep disturbance and socioeconomic status.[5-9] If left untreated, gestational diabetes may result in several maternal and fetal adverse outcomes.[2,6,9,10] Maternal adverse outcomes include preeclampsia, cesarean section, prolonged labor pain and miscarriages.[4,6-9] Whereas neonatal adverse outcomes include fetal macrosomia, neonatal metabolic disturbances (hypoglycemia), respiratory disorders, decreased 5-minute APGAR scores, neonatal intensive care unit (NICU) admission, impaired neurodevelopmental outcomes, autism spectrum disorder, polycythemia, hypocalcemia, jaundice, stillbirth and neonatal death.[2,4,6-11] These myriad of events was also reported in our local data by Malong et al., and Urbanozo et al.[12, 13] Other possible long-term adverse effects include obesity, metabolic syndrome, and diabetes of the child and the mother.[2,4,8-11] In fact, mothers

eISSN 2308-118x (Online)
Printed in the Philippines

Received: April 26, 2023. Accepted: July 27, 2023.
Published online first: January 23, 2024.
https://doi.org/10.15605/jafes.039.01.10

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with GDM are noted to have a seven-fold increase in developing type 2 diabetes.[10] In a tertiary hospital in Manila, the incidence of postpartum prediabetes and diabetes was 34.7% and 7.3%, respectively.[12]

With proper management of GDM, the risk of both maternal and fetal complications decreases and possibly improves health-related quality of life.[2,4,8,9] However, in some cases, there are no reduced risks, despite adequate medical treatment. This could be attributed to the need for a multidisciplinary approach in the management of GDM, which includes patient education on disease pathophysiology, compliance to medical treatment, disease monitoring to maintain normal glycemic levels and lifestyle modification that includes medical nutrition therapy, and physical activity.[2,4,9] Management of GDM is a labor-intensive discipline that poses several challenges to maintaining the highest quality of patient care.[4] But several impediments to the management of GDM exist such as the patient's low socioeconomic condition, inadequate knowledge, misbeliefs, myths, and social discrimination.[9] In a cross-sectional study done in Luzon, a higher level of education was a significant factor in the degree of self-efficacy and confidence to perform self-care (p = 0.023). In that same study, it also appeared that those who avail of free clinical services have better self-management practices (p <0.001).[14]

Health literacy is the measure of the patient's ability to read, understand and follow medical instructions.[2] Knowledge is one of the most vital components of health literacy. Insufficient health literacy is associated with poor knowledge about the disease, which would lead to poor adherence to management strategies.[9] According to studies, health literacy is of great importance in the management of complex chronic medical diseases, such as type 2 DM and human immunodeficiency virus (HIV) infection.[2] Individuals who do not know about their disease are less likely to comply with their treatment regimens as they do not understand its significance.[2,3,9] Appropriate knowledge about the disease accompanied with positive attitude and behavior, can help prevent complications of the disease through proper multidisciplinary management.[3,6] To prevent DM in two successive generations, patients with GDM will require thorough education that focuses on immediate care and their long-term health implications.[3,9] Patient education should be composed of information regarding normal and abnormal glycemic values, food restrictions, dietary values and the importance of exercise.[8] Hence, healthcare professionals must develop strategies to influence positive behavioral changes in these patients for them to adhere to proper exercise, diet and blood glucose monitoring.[6] It is therefore necessary to develop an efficient tool to reliably assess the diabetes knowledge of patients.[3]

Gestational Diabetes Mellitus Knowledge Questionnaire (GDMKQ) is a 15-item multiple-choice questionnaire that explores on the basic knowledge of GDM, risk factors, food and diet values, treatment options and management and complications or outcomes. This was developed by Hussain and colleagues in 2014 and was validated in Malaysia for knowledge assessment among pregnant patients with diabetes. Each item has 4 options with 1 correct answer. Each correct answer corresponds to 1 point. The score ranges from a maximum of 15 to a minimum of 0. Inadequate knowledge is indicated by a score ≤8 while a score >8 imparts adequate knowledge about GDM.[6,8]

In an extensive review of the literature and based on the researcher's knowledge, no local study has measured the patients' level of understanding of gestational diabetes. Identification of the domains which need improvement will help clinicians implement an effective learning program for use by pregnant women to improve health literacy and pregnancy outcomes.

This study aims to validate the Filipino-translated version of the Gestational Diabetes Mellitus Knowledge Questionnaire (GDMKQ) in evaluating the understanding of pregnant patients regarding gestational diabetes mellitus.

METHODOLOGY

Study design

This is a cross-sectional validation study conducted for a period of 2 months from January to February 2022 at the outpatient setting of Chinese General Hospital and Medical Center (CGHMC), Blumentritt, Manila.

Study population

A sample size of 45 participants was required in this study to have a ratio of 3 participants: 1 question item using the Andrew Fisher's formula with confidence level set at 95%. A total of 51 consecutive pregnant women were selected and enrolled in the study. Inclusion criteria were patients at least 18 years of age and with a clinical diagnosis of gestational diabetes mellitus (GDM) based on 75g OGTT with one or more of the following values: fasting plasma glucose ≥92 mg/dL (5.1 mmol/L), 1-hour plasma glucose ≥ 180 mg/dL (10 mmol/L), 2-hour plasma glucose ≥153 mg/dL (8.5 mmol/L). Participants must be able to read and understand both Filipino and English at a basic level. Those with pre-existing diabetes were excluded. All were assigned alphanumeric codes to hide their identity. Age, civil status, age of gestation, educational attainment, socioeconomic status, comorbidities, obstetric scores (gravidity and parity) and the date of initial diagnosis of GDM were documented in the data information sheet to ensure avoidance of missing data. Their contact numbers were collected to minimize loss to follow-up and address transfer bias.

This study protocol received approval from the Institutional Research and Ethics Review Board. Informed consent was obtained from each of the participants who agreed to be enrolled in the study.

Outcome measured

The primary outcome is the distribution of correct answers to each question, including the total and domain scores, while the secondary endpoint is the adequacy of knowledge of GDM.

Data collection and validation process

The Gestational Diabetes Mellitus Knowledge Questionnaire (GDMKQ) by Hussain et al. was used in this study. This was submitted to 4 healthcare professionals (2 Endocrinologists and 2 Obstetricians) who were all experts in GDM and fluent in both English and Filipino. One from each subspecialty performed the forward translation. The original version was not shown to the remaining medical experts who performed the backward translation to ensure an unbiased result. All of them gave their expert opinion on the comprehensibility of the questionnaire to improve its applicability. Clarifications of uncertainties were emailed to the principal investigator. The changes were collated and the revised questionnaire was sent back to the medical experts until a consensus was reached. The four medical experts evaluated the final forward-translated questionnaire. Following their recommendation, amendments were made to ensure its appropriateness for the target population.

Thirteen patients were then randomly selected for preliminary testing to ensure the readability and understandability of GDMKQ. This number was based on the article of Moore et al., wherein at least 12 participants are recommended for pilot studies.[15] Validation of the questionnaire involves validity measures such as content validity and concurrent validity and reliability measures like internal consistency and test-retest reliability. Content validity refers to the degree to which the questions adequately cover all the required content.[16] The translated questionnaire was pilot-tested on 13 actual patients with GDM for face validity. None reported any confusion about any of the items; hence, no further revisions were made.

Fifty-one eligible subjects seen in the out-patient clinics of Chinese General Hospital and Medical Center were given the self-administered GDMKQ questionnaire, in both Filipino and English translations, with a two-week interval in between. Concurrent validity and other reliability measures were then initiated.

Internal consistency is a measure of how reliable the items within a questionnaire are based on the intended construct. Statistically, this is computed by Cronbach's alpha and a value of at least 0.7 is considered statistically reliable. To ensure the reliability of answers, the Kuder-Richardson formula 20 (KR-20), which is based on the consistency of all responses to all items in the test, was used. Test-retest, which is statistically analyzed by Pearson's product-moment correlation coefficient *r* was performed. This gauges the consistency of a questionnaire by administering the same test over some time.[16] This was conducted by re-administering the GDMKQ Filipino version a month after the initial Filipino version over the same group of individuals. The average duration of completing each questionnaire was 5-8 minutes and those who completed all three questionnaires were included in the analysis.

Statistical analysis

SPSS v23 (IBM, Chicago, Illinois) was used in data analysis. Data were presented as mean ± standard deviation (SD) for continuous data following a normal distribution, as median (range) for discrete and highly skewed continuous data and as count (percent) for categorical data. Kolmogorov-Smirnov test for normality was used to determine the normal distribution of the variables. Age was noted to be normally distributed, while income and gestational age were skewed data. No missing data on patient characteristics were noted. The difference between the scores of the English version and the Filipino version of the questionnaire was determined using the student t-test. Independent t-test, Kuder-Richardson 20 (KR-20) and Pearson's product-moment correlation coefficient r were used to assess the validity and reliability of the questionnaire. $P \leq 0.05$ were considered statistically significant (Figure 1).

RESULTS

A total of 51 respondents were included and the demographic characteristics are summarized in Table 1. The mean age was 31.49 ± 6.445 years. The majority were married (60.8%) and college graduates (72.5%), with income classification ranging from poor to low middle-class income. The median gestational age was 27 weeks and most were multigravid (72.5%) and multiparous (37.3%). Some reported concomitant comorbidities including

Table 1. Baseline demographic characteristics of the GDM patients enrolled

Demographic data	n = 51
Age, in years (mean ± SD)	31.49 ± 6.445
Civil status, n (%)	
Single	20 (39.2%)
Married	31 (60.8%)
Level of education, n (%)	
Secondary / High School	14 (27.5%)
Tertiary / College	37 (72.5%)
Gravidity, n (%)	
Primigravida	14 (27.5%)
Multigravida	37 (72.5%)
Parity (median, range) n (%)	
Nullipara	14 (27.5%)
Primipara	18 (35.3%)
Multipara	19 (37.3%)
Gestational age, in weeks (median, range)	27 (18-38)
Monthly income (median, range)	₱13,000 (₱5,000- ₱35,000)
GDM management n (%)	
Diet-controlled	27 (53%)
On oral hypoglycemic agent	11 (21.6%)
On insulin	13 (25.4%)
Comorbidities n (%)	
Hypertension	4 (0.08%)
Dyslipidemia	2 (0.04%)
Hyperthyroidism	1 (0.02%)
Bronchial asthma	1 (0.02%)

hypertension (4), dyslipidemia (2), hyperthyroidism (1) and bronchial asthma (1).

Table 2 shows the distribution of correct answers in the English and Filipino versions of GDMKQ. Comparing the two questionnaires, correct answers were noted to be higher in the Filipino version, as well as the total scores and domain scores. The majority of the respondents have adequate knowledge of GDM in both versions of the questionnaire (English and Filipino, 82.4 and 86.3%, respectively) with a higher proportion in the Filipino version but not significantly different. Although there was an increase in the proportion with adequate knowledge, majority still had low knowledge on item 5 even after the re-test. In decreasing order, the scores among the five domains of the GDMKQ (both English and Filipino versions) are as follows: Knowledge about diet/food values > Basic knowledge of GDM > Knowledge about GDM complications > Knowledge about risk factors > Knowledge about GDM management.

Concerning civil status and educational attainment, an overall adequate knowledge of GDM was documented across all subgroups for both versions of GDMKQ. However, the total scores were higher in the married and college populations. The adequacy of their knowledge was higher in the Filipino version (52.9%, 66.7%) versus the English version (42%, 62.7%); meanwhile, the single and high school subgroup did not show a change. Between the two versions, those married and high school graduates scored higher in the Filipino version while the single and college graduates did better in English but were not significantly different. Better test scores were documented among college graduates than high school graduates in the English survey (11.24 ± 1.877 vs. 9.64 ± 2.951, $p = 0.025$) and Filipino retest survey (11.81 ± 1.664 vs. 10.07 ± 2.303, $p = 0.04$) while no significant difference was seen between different civil status. (Table 3)

The test-retest scores using the Filipino-translated questionnaire have a Pearson Correlation coefficient of 0.853 which showed a good positive correlation between the two scores ($p<0.001$). The Cohen's kappa was 0.564, while the intraclass correlation coefficient was 0.828, which means that there is a moderate to good level of agreement between the test scores. For reliability, the questionnaire yielded a KR-20 value of 0.632, which ranked strong (0.61 – 0.80), as shown in Table 4.

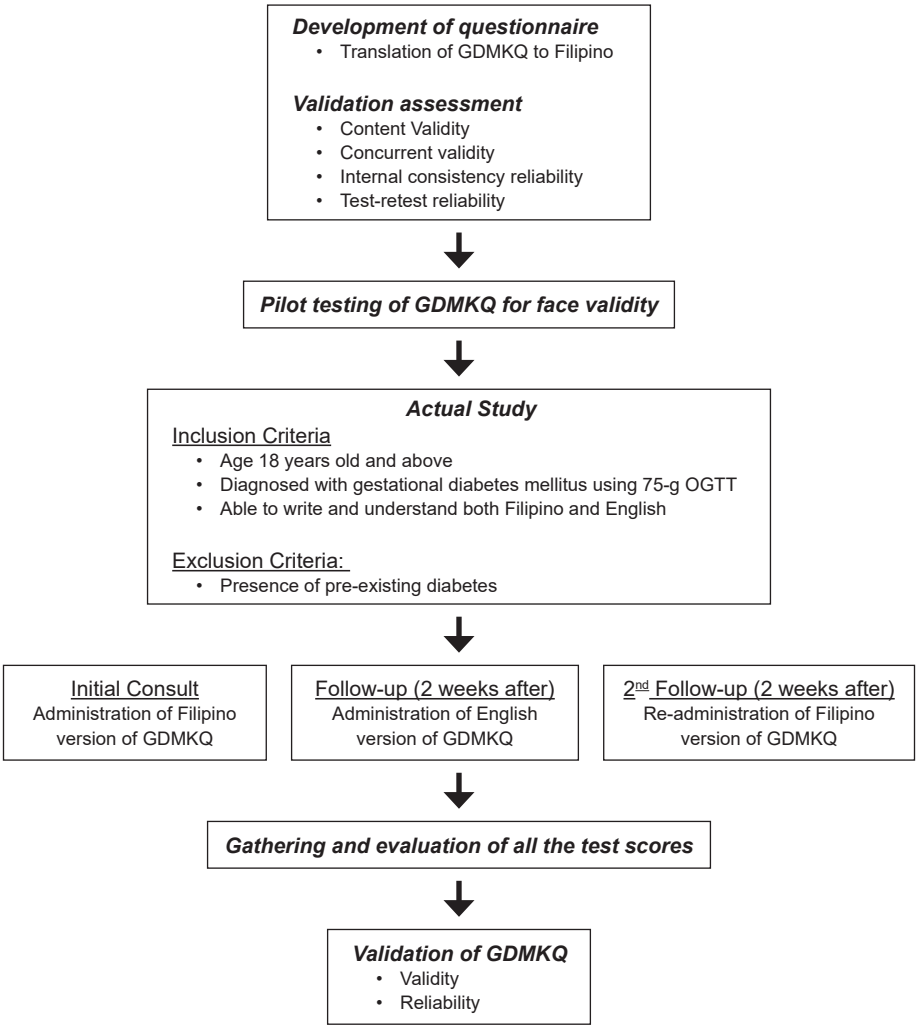

Figure 1. Overall recruitment flow.

Table 2. Distribution of correct answers among the English version and initial versus follow-up Filipino version in the GDMKQ questionnaire (n=51)

Domains/ Questions	English version Correct, n (%)	Initial Filipino version Correct, n (%)	Follow-up Filipino version Correct, n (%)
Basic knowledge about GDM			
Q1. Gestational Diabetes Mellitus is the type of diabetes that occur: **Correct: During pregnancy*	42 (82.4)	40 (78.4)	41 (80.4)
Q2. In uncontrolled Gestational Diabetes Mellitus, the blood sugar level is: **Correct: Increased*	43 (84.3)	49 (96.1)	49 (96.1)
Q3. What is the best way for testing blood glucose level for Gestational Diabetes Mellitus patients? **Correct: Blood test*	40 (78.4)	41(80.4)	42 (82.4)
Knowledge about risk factors			
Q4. You are at increased risk of developing Gestational Diabetes Mellitus if you are: **Correct: Overweight*	39 (76.5)	42 (82.4)	41 (80.4)
Q5. You have increased chances of developing Gestational Diabetes Mellitus if: **Correct: previously gave birth to a stillborn baby*	4 (7.8)	3 (5.9)	6 (11.8)
Q6. You are more likely to develop Gestational Diabetes Mellitus if you have: **Correct: Family history of diabetes*	41 (80.4)	40 (78.4)	43 (84.3)
Knowledge about diet/food values			
Q7. If you have Gestational Diabetes Mellitus, you should avoid food containing high content of: **Correct: carbohydrates and fats*	47 (92.2)	43 (84.3)	47 (92.2)
Q8. Which of the following food can be eaten without restriction during Gestational Diabetes Mellitus: **Correct: Fresh salad*	46 (90.2)	46 (90.2)	45 (88.2)
Q9. What is the type of nutritional source mainly provided by rice? **Correct: carbohydrates*	48 (94.1)	49 (96.1)	49 (96.1)
Knowledge about the management of GDM			
Q10. The most common sign of hyperglycemia (high blood sugar) is: **Correct: increased thirst*	23 (45.1)	28 (54.9)	33 (64.7)
Q11.The normal value of fasting blood sugar (FBS) is: **Correct: 3.6 – 6.1 mmol/l (64.8 - 109.8 mg/dL)*	30 (58.8)	25 (49)	29 (56.9)
Q12.If you feel the onset of hypoglycemic (low blood sugar) symptoms, you should: **Correct: Immediately eat or drink something sweet*	23 (45.1)	27 (52.9)	30 (58.8)
Knowledge about GDM complications			
Q13. In uncontrolled Gestational Diabetes Mellitus, your baby may be: **Correct: Larger than usual size*	34 (66.7)	37 (72.5)	35 (68.6)
Q14. If you have Gestational Diabetes Mellitus you have: **Increased chances of developing diabetes in later life*	39 (76.5)	37 (72.5)	41 (80.4)
Q15. Gestational Diabetes Mellitus is a condition that: **Correct: May affect mother or baby*	48 (94.1)	49 (96.1)	51 (100)
Total score:	**551**	**553**	**577**
Average score:	**10.80**	**10.84**	**11.31**

DISCUSSION

This is the first Filipino-validated study of GDMKQ. Worldwide, the prevalence of gestational diabetes complicating pregnancy approaches 20%.[5] Based on literature searches, not much attention has been given to the assessment of understanding of this condition, as compared to type 1 and type 2 diabetes. In the study of Malong et al., the incidence of diabetes and prediabetes postpartum was 7.3% and 34.7% respectively; hence, the importance of vigilance in peripartum care.

The GDMKQ underwent three revisions until a consensus was reached. The medical experts simplified some of the terms to make the questionnaire more comprehensible and relevant. In the original GDMKQ, question 12 reads "If you feel the onset of hypoglycemia (low blood sugar) symptoms, you should" and the options were listed. Since hypoglycemia has several symptoms, one of the panelists deemed that examples of it should be stated. They also suggested placing conventional units aside from the SI units since some patients may be more acquainted with the other unit of measurement. One of them also suggested keeping the term "stillbirth" and place it beside its Filipino translation. All the suggestions of the medical experts were implemented to ensure acceptability across ethnicities. For the pilot testing, thirteen participants were randomly selected. While all participants agreed that the GDMKQ was easy to understand, majority incorrectly answered question 5 and claimed that it was the first time they learned that a previous "stillbirth" was a risk factor for developing GDM.

The study showed that the majority (86.3%) had adequate knowledge of GDM as demonstrated by the number of correct answers and total scores. This was exhibited across all subgroups regardless of civil status and educational attainment but was particularly found among married and college graduates. The significant increase in this proportion is because their number was higher upon enrollment as opposed to their counterparts (31 vs 20; 37 vs 14, respectively) and the good knowledge is likely due to the counseling done by the primary physician during prenatal visits even before their recruitment. The knowledge adequacy of these respondents was higher in the Filipino version of GDMKQ, likely because Filipino is

Table 3. Percentages of correct and incorrect scores for questions in each domain of the GDMKQ (n=51)

GDMKQ	English version		Initial Filipino version		Follow-up Filipino version	
	Correct, n (%)	Incorrect, n (%)	Correct, n (%)	Incorrect, n (%)	Correct, n (%)	Incorrect, n (%)
Domain 1: Basic knowledge of GDM						
Q1	42 (82.4%)	9 (17.6%)	40 (78.4%)	11 (21.6%)	41 (80.4%)	10 (19.6%)
Q2	43 (84.3%)	8 (15.7%)	49 (96.1%)	2 (3.9%)	49 (96.1%)	2 (3.9%)
Q3	40 (78.4%)	11 (21.6%)	41 (80.4%)	10 (19.6%)	42 (82.4%)	9 (17.6%)
Average score:	41.67		43.3		44	
Domain 2: Risk factors						
Q4	39 (76.4%)	12 (23.5%)	42 (82.4%)	9 (17.6%)	41 (80.4%)	10 (19.6%)
Q5	4 (7.8%)	47 (92.2%)	3 (5.9%)	48 (94.1%)	6 (11.8%)	45 (88.2%)
Q6	41 (80.4%)	10 (19.6%)	40 (78.4%)	11 (21.6%)	43 (84.3%)	8 (15.7%)
Average score:	28		28.3		30	
Domain 3: Food and diet values						
Q7	47 (92.2%)	4 (7.8%)	43 (84.3%)	8 (15.7%)	47 (92.2%)	4 (7.8%)
Q8	46 (90.2%)	5 (9.8%)	46 (90.2%)	5 (9.8%)	45 (88.2%)	6 (11.8%)
Q9	48 (94.1%)	3 (5.9%)	49 (96.1%)	2 (3.9%)	49 (96.1%)	2 (3.9%)
Average score:	47		46		47	
Domain 4: Treatment options and management						
Q10	23 (45.1%)	28 (54.9%)	28 (54.9%)	23 (45.1%)	33 (64.7%)	18 (35.3%)
Q11	30 (58.8%)	21 (41.2%)	25 (49%)	26 (51%)	29 (56.9%)	22 (43.1%)
Q12	23 (45.1%)	28 (54.9%)	27 (52.9%)	24(47.1%)	30 (58.8%)	21 (41.2%)
Average score:	25.3		26.67		30.67	
Domain 5: Complications or outcomes						
Q13	34 (66.7%)	17 (33.3%)	37 (72.5%)	14 (27.5%)	35 (68.6%)	16 (31.4%)
Q14	39 (76.5%)	12 (23.5%)	37 (72.5%)	14 (27.5%)	41 (80.4%)	10 (19.6%)
Q15	48 (94.1%)	3 (5.9%)	49 (96.1%)	2 (3.9%)	51 (100%)	0 (0%)
Average score:	40.3		41		42.3	
Knowledge Score, median	11 [IQR: 10-12]		11 [IQR: 10-12]		11 [IQR: 10-13]	
Adequate, n (%)	42 (82.4%)		44 (86.3%)		44 (86.3%)	
Inadequate, n (%)	9 (17.6%)		7 (13.7%)		7 (13.7%)	

Table 4. Comparison of total scores according to civil status and educational attainment

	n	English version			Filipino version					
					Initial			Follow-up		
		Knowledge scores	Comparison of test scores	Between-group *P*	Knowledge scores	Comparison of test scores	Between-group *P*	Knowledge scores	Comparison of test scores	Between-group *P*
Civil status										
Single	20	214	10.70 ± 1.949	0.799	213	10.65 ± 2.084	0.585	224	11.20 ± 2.118	0.706
Adequate, n (%)		17(33.3%)			17 (33.3%)			17 (33.3%)		
Inadequate, n (%)		3 (5.9%)			3 (5.9%)			3 (5.9%)		
Married	31	337	10.87 ± 2.54		340	10.97 ± 1.975		354	11.42 ± 1.945	
Adequate, n (%)		25 (49%)			27 (52.9%)			28 (54.9%)		
Inadequate, n (%)		6 (11.8%)			4 (7.8%)			3 (5.9%)		
Educational attainment										
High school	14	135	9.64 ± 2.951	0.025*	140	10 ± 2.353	0.064	141	10.07 ± 2.303	0.004*
Adequate, n (%)		10 (19.6%)			10 (19.6%)			9 (17.6%)		
Inadequate, n (%)		4 (7.8%)			4 (7.8%)			5 (9.8%)		
College	37	416	11.24 ± 1.877		413	11.16 ± 1.788		437	11.81 ± 1.664	
Adequate, n (%)		32 (62.7%)			34 (66.7%)			36 (70.6%)		
Inadequate, n (%)		5 (9.8%)			3 (5.9%)			1 (2%)		

[a]Wilcoxon signed-rank test was used; [b]Independent t-test was used; One Way ANOVA

our basic and national language. Those who were single and high school graduates, on the other hand, did not demonstrate change for both versions of GDMKQ and re-test of the Filipino version.

Between the two versions, total scores were found to be higher in the English GDMKQ among the single and college graduates while those married and high school graduates fared better in the Filipino version. A possible explanation would be more than half (65%) of those who were single were college graduates and English is the medium of instruction in the academe and universities. However, no significant difference has been found between the English and Filipino versions of GDMKQ (p = 0.834). The scores in both versions were almost the same, which means that the translated version is valid.

In decreasing order, the frequency of correct responses among the five domains of gestational diabetes is as follows: knowledge about diet and food values > basic knowledge about GDM > knowledge about GDM complications > knowledge about risk factors > knowledge about management of GDM. Only limited studies can be used for the comparison of these findings, as there were

only three studies examining the validity and reliability of GDM knowledge questionnaires have been found.[16] Alayoub et al., Hussain et al., and Ogu et al., developed a questionnaire on knowledge assessment but no one elaborated on the validation process.[6,8,17,18] The high literacy on diet and food values documented in this study, along with that of Hussain's, may be explained by the greater number of patients who were diet-controlled and did not require medications.[6,8] On the other hand, the domain with the lowest frequency of correct answers was GDM management. This finding is likely due to the little emphasis on self-management principles and lockdown implementation during the surge of COVID-19 disease which limited the patient's follow-up consult. As opposed to the finding of Carolan-Olah, this domain garnered the highest response in Australia, where the study was done, which may be due to its economic status as a developed country.[16]

For the test-retest reliability (Tables 5 and 6), a coefficient of stability of 0.853 was obtained, indicating a very high correlation with a moderate to good level of agreement as reflected by the computed Cohen's kappa of 0.564 and intra-class correlation coefficient of 0.828. When analyzing the internal consistency of all test items of the GDMKQ, a good internal consistency (KR-20 = 0.632) was identified but was noted to be lower than the findings of Carolan-Olah which reported Cronbach's alpha coefficient (α = 0.88). Nevertheless, both signify good internal consistency or reliability as KR-20 values of at least 0.6 (Table 6) and Cronbach's coefficient of at least 0.7 are considered satisfactory.[16]

The retest scores were also found to be significantly higher than the initial test ($p = 0.002$) which is likely due to patient education that occurred within the period of follow-up. Among the respondents, those with a tertiary level of education aced the questionnaire as shown in Table 3. This is in accordance with the statement of Spoelman that those with higher education levels perform more health searches on the internet or websites and, thus, are likely to achieve a higher score.[19]

Some limitations recognized by the researchers include the use of Andrew Fisher's formula in the sample size, lack of quantitative assessment of content validity and self-selection bias. The evaluation of content validity is a subjective process. The majority of women who participated had a tertiary level of education which was also seen in the study of Carolan-Olah.[16] According to studies, women with higher levels of education are more likely to participate.[19] They have increased access to health-related literature, books and internet sources, hence portending a higher knowledge score.[8] Rasch analysis can be used in future assessments to evaluate the different personal qualities such as education of respondents. Secondly, the participants have been recruited within the hospital, hence, they tend to possess better health knowledge and better health-seeking behavior. Lastly, this is only a single-centered trial which may not reflect the overall patient

Table 5. Reliability analysis using Kuder Richardson-20 item analysis

	Scale mean if item deleted	Scale variance if item deleted	Corrected item - total correlation	KR-20 if item deleted
Q1	30.45	24.093	0.061	0.637
Q2	29.63	23.678	0.519	0.618
Q3	31.29	24.492	-0.050	0.657
Q4	31.27	21.563	0.259	0.615
Q5	29.37	20.598	0.328	0.602
Q6	31.12	20.506	0.300	0.608
Q7	30.53	23.774	0.098	0.634
Q8	31.41	21.767	0.296	0.609
Q9	30.59	23.447	0.291	0.618
Q10	29.98	21.140	0.389	0.594
Q11	30.04	24.038	-0.040	0.671
Q12	29.86	20.601	0.407	0.589
Q13	30.96	18.118	0.490	0.563
Q14	30.24	20.064	0.531	0.570
Q15	30.59	23.447	0.291	0.618

Table 6. Summary of reliability analysis done

Summary of reliability analysis

Test statistics	Value	Interpretation
Cohen's kappa	0.564	Moderate strength of agreement
ICC overall	0.632	Substantial agreement
ICC domain 1	0.149	Slight agreement
ICC domain 2	0.433	Moderate agreement
ICC domain 3	0.079	Slight agreement
ICC domain 4	0.505	Moderate agreement
ICC domain 5	0.497	Moderate agreement
Kuder Richardson 20	0.632	Moderate correlation, substantial agreement

Kuder-Richardson coefficient of reliability rank

Reliability coefficient	Level of reliability
0.81 or more	Near complete agreement
0.61 – 0.80	Strong
0.41 – 0.60	Moderate
0.21 – 0.40	Fair
0.00 – 0.20	Poor agreement

characteristics. Addressing these factors is necessary and a multi-center study is recommended.

CONCLUSION

The Filipino-translated version of GDMKQ is a valuable tool in evaluating the knowledge of women on gestational diabetes. This can be used as a simple screening device in the outpatient setting to recognize the facts and misconceptions of pregnant women about GDM and its management. Therefore, clinicians will be able to develop an individualized, effective learning program that will help mitigate the risks and improve pregnancy outcomes.

Acknowledgments
The authors wish to express their sincere gratitude to the panel of experts who evaluated the questionnaire, Dr. Francis Xavier Mislang, Dr. Maria Guia Estrella Dela Cruz, Dr. Jonie Tan and Dr. Vicar Ong. Gratitude is also extended to the patients who consented to participate and to the consultants of the Section of Endocrinology, Diabetes and Metabolism of the Chinese General Hospital and Medical Center for their continued support for this research.

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

CRediT Author Statement
HG: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **FRG:** Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing – review and editing, Supervision

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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Journal of the ASEAN Federation of Endocrine Societies

ORIGINAL ARTICLE

Fructosamine and HbA1c: A Correlational Study in a Southeast Asian Population

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Abstract

Objectives. Fructosamine correlates well with glycated haemoglobin (HbA1c) in Caucasians. This study investigates this correlation and whether fructosamine can reliably estimate glycated haemoglobin in Southeast Asians.

Methods. We recruited 193 participants based on 4 HbA1c bands (<6.0%; 6.0 – 7.9%; 8.0– 9.9%; ≥10%) from a secondary hospital in Singapore between August 2017 and December 2021. Blood samples for fructosamine, glycated haemoglobin, albumin, haemoglobin, thyroid stimulating hormone and creatinine were drawn in a single setting for all participants. Scatter plot was used to explore correlation between fructosamine and glycated haemoglobin. Strength of linear correlation was reported using Pearson's correlation coefficient. Simple linear regression was used to examine the relationship between fructosamine and glycated haemoglobin.

Results. We performed simple linear regression to study the relationship between fructosamine and HbA1c in the research participants (R^2 = 0.756, p<0.01). Further analysis with natural logarithmic transformation of fructosamine demonstrated a stronger correlation between HbA1c and fructosamine (R^2 = 0.792, p<0.01).

Conclusions. Fructosamine is reliably correlated with HbA1c for the monitoring of glycaemic control in Southeast Asians.

Key words: fructosamine, glycated haemoglobin, Southeast Asian population, diabetes mellitus

INTRODUCTION

Hyperglycaemia promotes non-enzymatic glycation of proteins through the Maillard reaction with Schiff base formation through Amadori rearrangements.[1] Glycated haemoglobin A1c (HbA1c) is formed when the N-terminal valine residue of the beta chain is glycated.[2] The half-life of HbA1c is 4 weeks[3] and it reflects average blood glucose over the past 1 to 4 months. It was estimated that 50% of HbA1c reflects blood glucose in the past 1 month, 25% for the past 1 to 2 months, and 25% for the past 2 to 4 months.[4] Hence, HbA1c does not accurately reflect blood glucose in the immediate period before blood sampling. The correlation between HbA1c and blood glucose is further eroded in the setting of pregnancy, advanced kidney disease, and conditions altering erythrocyte life expectancy, such as haemoglobin variants, haemoglobinopathies, and iron deficiency.[5–9]

Glycation of plasma proteins form ketoamines. These are collectively known as fructosamine because of the 1-amino-1-deoxy-D-fructose groups that are present in the glycated protein molecules. The half-life of fructosamine is 2.5 weeks[10,11] and it reflects blood glucose in the preceding 2 to 4 weeks.[12] Thus, fructosamine provides better reflection of blood glucose in the shorter term compared to HbA1c. Fructosamine had similar diagnostic performance as fasting glucose in detecting diabetes in a North American population.[13] Fructosamine was also found to predict microvascular complications of diabetes.[14–16] It is reportedly more suitable in monitoring blood glucose[17] and predicted stillbirths in pregnant women with diabetes.[18] One benefit of using fructosamine as a marker of glycaemic control is that it is not affected by erythrocyte lifespan or haemoglobin structure[12] as compared to HbA1c. This is of particular importance in Southeast Asia where iron deficiency and haemoglobin variants are highly prevalent. Southeast Asia was ranked as the region with the highest prevalence for iron deficiency anaemia in 2010.[19] The prevalence of iron deficiency in Malaysian school children was reported to be between 4.4% and 5.2%[20,21] while up to 74% of Singaporean women in the third trimester of pregnancy

eISSN 2308-118x (Online)
Printed in the Philippines

Received: May 25, 2023. Accepted: August 4, 2023.
Published online first: February 8, 2024.
https://doi.org/10.15605/jafes.039.01.14

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were iron deficient in a cross-sectional study.[22] Southeast Asia was reported in 2010 as one of the regions with the highest prevalence of thalassaemias.[19] Between 4.5% and 22.6% of Malaysians were estimated to carry the gene for alpha thalassaemia.[23] Between 1 and 9% of Southeast Asians carry the beta thalassaemia gene and between 1 and 8% carry the gene for Haemoglobin Constant Spring.[24] Fructosamine was found to correlate well with HbA1c in studies conducted in North America,[15] Sweden,[25] Spain,[26] Korea[27] and the United Kingdom,[3] involving participants with and without diabetes. Considering that fructosamine correlates well with HbA1c under normal circumstances and that erythrocyte abnormalities are highly prevalent in Southeast Asia, it can potentially be used as an alternative to HbA1c to monitor glycaemic status in patients with erythrocyte disorders. Thus, we aimed to find out if this correlation exists in a Southeast Asian population and whether fructosamine can reliably estimate HbA1c.

METHODOLOGY

Recruitment

This is a cross-sectional study. Patients were recruited from the Diabetes outpatient clinic, and the Health and Wellness clinic of Ng Teng Fong General Hospital between August 2017 and December 2021 based on 4 HbA1c bands: <6.0%; 6.0–7.9%; 8.0–9.9%; ≥10%. The sampling design is by purposive sampling through the recruitment of patients in the clinic, to ensure all ranges of HbA1c are represented equally.

Inclusion criteria

The inclusion criteria include all the following: people without diabetes based on medical history or physical examination record, patients with type 1 or type 2 diabetes, age between 21 and 99 years, of Chinese, Indian, or Malay ethnicity and who are able to provide informed consent.

Exclusion criteria

The exclusion criteria include any of the following: pregnant women, patients with erythrocyte disorders (defined as mean corpuscular volume above or below the normal limits), anaemia (defined as haemoglobin below the lower limit of normal), protein-losing disorders, thyroid disorders, estimated glomerular filtration rate below 60 mL/min, on erythropoietin therapy or who have received blood transfusion within the preceding three months of recruitment.

Analytical methods

The following were measured based on either fasting or random serum samples from each participant during a single visit in a seated position: HbA1c, fructosamine, albumin, creatinine, full blood count and thyroid stimulating hormone (TSH).

Samples for HbA1c were collected in ethylenediaminetetraacetic acid tubes and measured using an enzymatic assay (Abbott Architect c8000, interassay coefficient of variation (CV) ≤2% when HbA1c is between 5.7% (39 mmol/mol) and 7.0% (53 mmol/mol), ≤3.5% when HbA1c >7.0% (53 mmol/mol). This method is standardised to the Diabetes Control and Complications Trial assay. Samples for fructosamine were collected in plain tubes and measured using spectrophotometry (Roche Cobas c502, total imprecision when fructosamine < 285 umol/L is ≤9 umol/L, CV ≤3% when fructosamine >285 umol/L). Samples for albumin were collected in serum separator tubes and measured using the photometric method based on binding with bromcresol green (Abbott Architect c16000, CV ≤3.3%). Samples for creatinine were collected in serum separator tubes and measured using the enzymatic method (Abbott Architect c16000, CV ≤3.6%). Samples for haemoglobin were collected in ethylenediaminetetraacetic acid tubes. Samples for TSH were collected in serum separator tubes and measured using a chemiluminescent microparticle immunoassay (Abbott Architect i2000SR, CV ≤10%).

The laboratory results were recorded and secured in the hospital's electronic medical record system.

Sample size calculation

The sample size of 203 was calculated based on 80% statistical power and 5% Type I error rate. We assumed a modest positive linear correlation of 0.5 between HbA1c and fructosamine for each of the three ethnic groups (Chinese, Malay, and Indian) and 4 pre-defined HbA1c bands.

Statistical analysis

SPSS version 28 was used for the statistical analysis. Descriptive statistics was used to summarise the demographics of participants. Scatter plot was used to explore the relationship between fructosamine and HbA1c. Strength of linear correlation was reported using Pearson's correlation coefficient. A stronger correlation between nature log transformed fructosamine and HbA1c than the untransformed fructosamine was observed and it indicated that HbA1c might be linearly associated with log-transformed fructosamine. Simple linear regression model was used to evaluate the linear relationship between nature log transformed fructosamine and HbA1c in the research participants. Assumptions of linear regression model were checked by the residual plots to ensure the model's validity. R^2 was reported to evaluate the fitness of the model. A *p*-value less than 0.05 was considered statistically significant.

Ethical concerns

The study was approved by the Domain Specific Review Board of the National Healthcare Group (Singapore). Signed informed consent was obtained from all research participants. All study data were de-identified.

RESULTS

Characteristics of research participants

One hundred and ninety-three research participants were recruited and analysed (Figure 1). Recruitment stopped at 193 participants after analysis demonstrated $R^2 = 0.792$. The characteristics of research participants with regards to gender, ethnicity, age and biochemical parameters are summarised in Table 1. Based on 4 HbA1c bands: <6.0%; 6.0 – 7.9%; 8.0 – 9.9%; ≥10%; there were 50 (25.9%), 50 (25.9%), 47 (24.4%) and 46 (23.8%) participants recruited in the respective categories.

Relationship between fructosamine and HbA1c

We performed simple linear regression to study the relationship between fructosamine and HbA1c in the research participants ($R^2 = 0.756$, $p<0.01$) (Figure 2). Further analysis with natural logarithmic transformation of fructosamine demonstrated a stronger correlation between HbA1c and fructosamine ($R^2 = 0.792$, $p<0.01$). Linear relationship between Ln fructosamine and HbA1c is shown in the scatter plot (Figure 3). This correlation study yields the following:

(HbA1c is expressed in % and fructosamine is expressed in umol/L):

$$\text{HbA1c} = (\text{Ln Fructosamine} \times 8.14) - 38.82$$

Table 2 lists the estimated corresponding fructosamine values for HbA1c based on the correlation model.

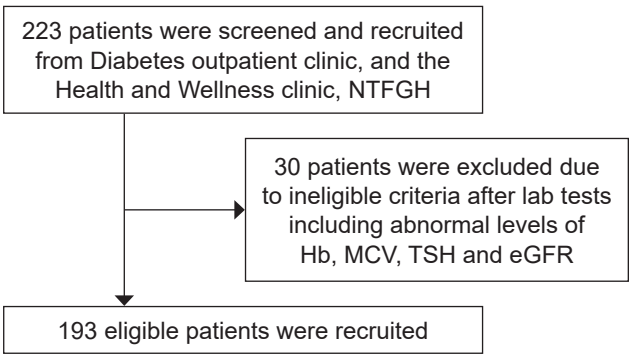

Figure 1. Overall recruitment flow.

Table 1. Characteristics of research participants

Characteristic	All
Female, n (%)	86 (44.6%)
Male, n (%)	107 (55.4%)
Age (years, mean, SD)	53.7, 12.7 Range: 22-76
Chinese, n (%)	125 (64.8%)
Indian, n (%)	42 (21.8%)
Malay, n (%)	26 (13.5%)
Hb (g/dL, mean, SD)	14.3, 1.4
MCV (fL, mean, SD)	86.0, 3.5
Albumin (g/L, mean, SD)	42.7, 2.5
Estimated glomerular filtration rate (mL/min, mean, SD)	98.1, 13.2
HbA1c (%, mean, SD)	8.0, 2.2
HbA1c (mmol/mol, mean, SD)	64, 24
Fructosamine (umol/L, mean, SD)	325.9, 83.4

n: number; SD: standard deviation

Table 2. Interpretation of correlation model

HbA1c (%)	HbA1c (mmol/mol)	Fructosamine (umol/L)
6.5	47.5	261.8
7.0	53.0	278.4
7.5	58.5	296.0
8.0	63.9	314.8
8.5	69.4	334.7
9.0	74.9	355.9
9.5	80.3	378.5
10.0	85.8	402.4

DISCUSSION

We demonstrated a strong correlation between fructosamine and HbA1c in Southeast Asian patients, and this is similar to prior studies in Caucasian populations.[3,25,26] HbA1c may 'average' out the highs and lows of glucose control for the past 4 months while fructosamine values would only reflect the levels in the preceding month. Furthermore, protein glycation via the Maillard reaction depends on other factors such as the availability and reactivity of amino groups on proteins, intracellular and extracellular glucose concentration, concentration and reactivity of carbonyl compounds and the rate of deglycation and elimination of Maillard products.[28] These factors may explain the variability in fructosamine levels for the same HbA1c level. We believe this variability is unlikely to have a major impact on using fructosamine to monitor glycaemic control because there was strong correlation between fructosamine and HbA1c in our research participants.

Our study suggests that fructosamine can be used as a reliable adjunct to HbA1c in monitoring glycaemic control in a Southeast Asian population.

This is the first study on the relationship between fructosamine and HbA1c in a Southeast Asian population. Laboratory analyses were done on fresh blood samples using validated techniques so the chance of erroneous measurements is low. We excluded patients with thyroid disorders because serum protein turnover is lower in hypothyroidism and higher in hyperthyroidism, which leads to increased and decreased protein glycation respectively.[29,30] We also excluded patients with protein-losing disorders. Hence, the rates of serum protein turnover and glycation in the study participants were likely to be occurring at steady states.

There are limitations of this study arising from the characteristics of participants. The age of participants is between 22 and 76 years (mean age = 53.7 years) and children were excluded from this study. Thus, it is uncertain whether a similar correlation exists in the paediatric or older Southeast Asian population. However, a correlation was demonstrated in an older Caucasian cohort (mean age = 70

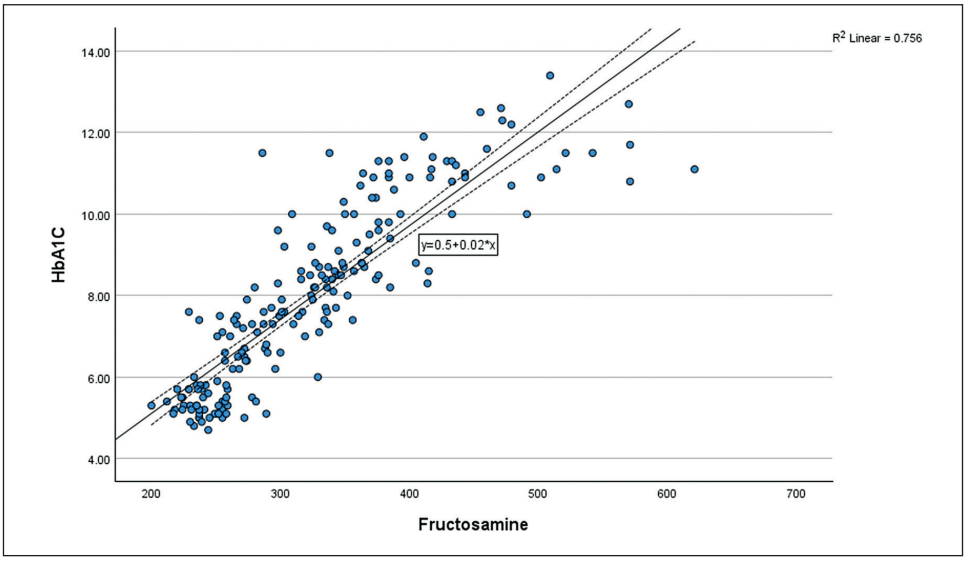

Figure 2. Scatter plot of Fructosamine against HbA1c.

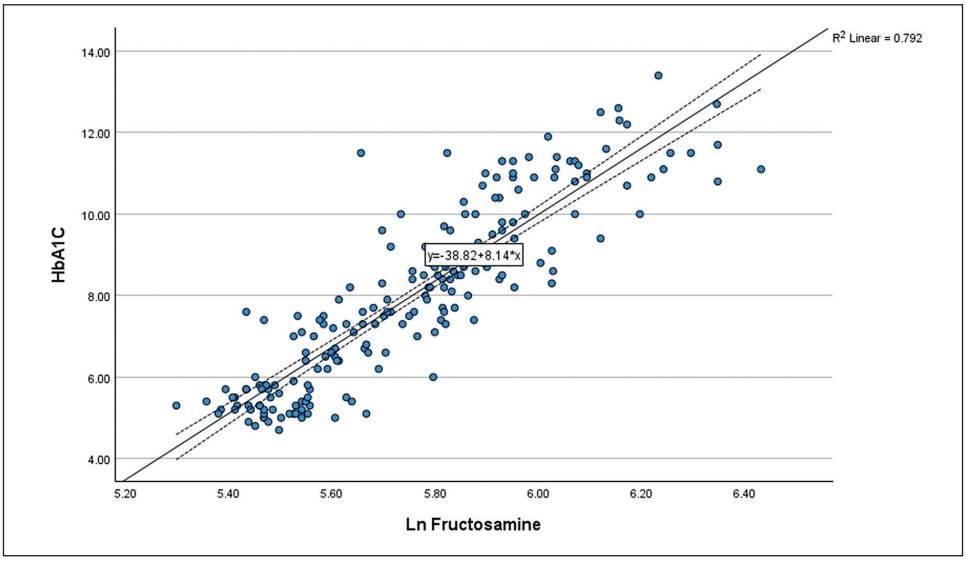

Figure 3. Scatter plot of Ln Fructosamine against HbA1c.

years)[13] and in a paediatric cohort in the United Kingdom.[3] In addition, patients with anaemia, haemoglobinopathies and those who were pregnant were excluded from this study. Theoretically, fructosamine levels would be a better indicator of glycaemic status in such patients. In patients with protein-losing disorders, the use of fructosamine may be less than ideal. The extent of HbA1c variability with fructosamine levels in these patients could be a subject of further investigation.

This study has limitations arising from the cross-sectional nature of its design. We were unable to conduct sub-group analysis based on differences in age, gender, ethnicity, or recent changes in diabetes medications because of the small sample size. We were unable to account for confounding factors arising from poor adherence to diabetes medication, or deviations in dietary habits or activity levels in this observational study.

CONCLUSION

In conclusion, we demonstrated a strong correlation between fructosamine and HbA1c in a multi-ethnic Southeast Asian population. Fructosamine can be considered as a reliable alternative to HbA1c in monitoring glycaemic control.

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

CRediT Author Statement
KC: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **SML:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review and editing, Visualization, Project administration; **LS:** Software, Validation, Formal analysis, Data curation, Visualization; **ELT:** Conceptualization, Methodology, Validation, Formal analysis,

Investigation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision.

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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ORIGINAL ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

The Acute Coronary Syndrome Risk in Medically Managed Subjects with Type 2 Diabetes Mellitus – Is the ASCVD Risk Score Failing Here?

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Abstract

Objectives. Type 2 Diabetics have elevated risk for acute coronary syndrome (ACS). The current management algorithm focuses on atherosclerotic cardiovascular (ASCVD) risk score to stratify this risk. However, in medically managed subjects, this algorithm may not be accurate. This study compares the ASCVD risk score in an Indian population with T2DM under medical supervision and the actual incidence of ACS. It also compared the ASCVD risk scores in cases with T2DM who developed ACS to controls and tried to estimate whether the ASCVD risk score is different in the two subsets, evaluating the utility of the ASCVD risk score in predicting ACS.

Methodology. This is an electronic medical record (EMR) based case-control study. Only records of subjects with T2DM where details of age, sex, body mass index, blood pressure, duration of diabetes, family history of ACS, lipid profile, renal and liver function tests were included. The incidence of ACS was calculated in the selected records, and the records of subjects with ACS were compared with age and sex-matched subjects without ACS. Data are summarized as median and interquartile range (IQR). Wilcoxon rank-sum test was used for checking differences in continuous variables and Pearson's Chi-squared test for categorical data. Univariate and multivariate logistic regression analyses were used to check the effect of ASCVD scores and other variables on the occurrence of ACS.

Statistical data analyses were performed using JASP, version 0.16.4 (JASP Team [2022]) for MS Windows.

Results. Of the 1226 EMRs included in the analysis, 207 had ACS. The actual incidence of ACS was 16.85% in 6 years, higher than the mean predicted 10-year incidence of 14.56 percent (p <0.05). The cases were age and sex-matched with controls and the ASCVD incidence was estimated in the two groups. The mean ASCVD score in the cases was 14.565 ± 8.709 (Min: 1.5, Max: 38.3) and controls 13.114 ± 8.247 (Min: 1.4, Max: 45). The chance of development of ACS increases with elevated systolic blood pressure (per mmHg rise OR: 1.04, 95% CI: 1.03, 1.06; p <0.001), positive family history (OR: 5.70, 95% CI: 3.41, 9.77; p <0.001), statin use (OR: 2.26, 95% CI: 1.46, 3.52; p <0.001), and longer duration of diabetes (for every year increase OR: 1.19, 95% CI: 1.13, 1.25; p <0.001).

Conclusion. The ASCVD risk score underestimates the ACS risk in subjects with T2DM under medical supervision and may not differ in those who developed and did not develop ACS. We also conclude that factors like a negative family history (30% less risk), longer duration of diabetes, and higher SBP are relevant in those who developed ACS.

Key words: ASCVD, acute coronary syndrome, family history

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is reaching epidemic proportions across the globe with the International Diabetes Federation estimating that close to 537 million people are living with diabetes in 2021. India is home to 74 million people with T2DM. Ischaemic heart disease (IHD) affects almost one-third of people with diabetes and is the leading cause of mortality accounting for close to 9 million deaths per annum. T2DM is a major risk factor for IHD and ACS which is almost 2-3 times common in people with T2DM as compared to controls.[1,2]

T2DM is ironically a silent disease existing long before it is symptomatic. It also remains the main reason responsible for the leading cause of mortality that is atherosclerotic

eISSN 2308-118x (Online)
Printed in the Philippines

Received: June 26, 2023. Accepted: September 1, 2023.
Published online first: February 5, 2024.
https://doi.org/10.15605/jafes.039.01.15

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cardiovascular disease (ASCVD). The existence of metabolic derangements like dysglycaemia, hypertension and dyslipidaemia precede the development of ASCVD. These remain undiagnosed for a long time before ASCVD manifests. Many attempts have been made in the past to do risk stratification for ASCVD. Most of these were based on the existence of risk factors like age, sex, smoking, diabetes, hypertension, dyslipidaemia, etc.[2-4]

The main utility of these risk engines has been for the identification of individuals who are to be targeted for therapies, most notably statins, for the prevention of ASCVD. However, time and again, some limitations of these risk engines have been realised like the non-incorporation of family history of ASCVD as well as the omission of factors like obesity. This may be responsible for the fact that even in countries with better health coverage, the residual risk of ASCVD remains to be addressed. The utility of these risk engines, in particular, ASCVD risk score in the assessment of risk in T2DM is not yet proven. Also, in those subjects with T2DM who are in regular follow-up with their physicians as well as on medical management for diabetes, hypertension, and dyslipidaemia, the utility of ASCVD risk score to predict ACS risk is not well studied.[4]

The target study population for the generation of these risk engines has been Caucasians and so, the validity of these in other races is doubtful. Indian or Southeast Asian population is different in that the onset of ASCVD is quite early as compared with Caucasians.[5] Also, the pattern of obesity in Indians is predominantly central with metabolically unhealthy abdominal fat which exists even despite body mass indices that may fall within the normal range as per Caucasian standards.[5,6]

The most common risk score used to predict future cardiovascular risk is the Atherosclerotic Cardiovascular Disease (ASCVD) risk score 2013 by the American Heart Association. Data from diverse racial populations were used to get the ASCVD risk score. This score estimates the cardiovascular risk based on variables like age, sex, race, total cholesterol levels, HDL levels, blood pressure, history of diabetes and hypertension, addictions like smoking and chewing tobacco and use of medications like aspirin and statin. Patients are categorized as high (>20%), intermediate (7.5-20%), borderline (5-7.5%) or low (<5%) risk depending on the score calculated. ASCVD risk score is the maximally used risk score to estimate the possible cardiovascular event risk in clinical practice and is used to make management decisions based on the risk obtained.[7]

However, the ASCVD risk score validity has not been studied in a population which is under regular medical follow-up. This population is different because they are regularly on medications for diabetes, hypertension, and dyslipidaemia as well as other comorbidities. The main objective of this study was, therefore, to look at the performance of ASCVD risk score in people with T2DM on medical management as well as to compare the ASCVD risk score in people with T2DM who developed ACS with those age- and sex-matched T2DM who did not. The secondary objective of the study was to look at the impact of factors like blood pressure, lipid levels, family history of diabetes, duration of diabetes and smoking status on the development of ACS.

OBJECTIVES

Primary objectives

The primary objective was to assess whether the ASCVD risk calculator accurately estimates the ACS risk in people with T2DM under medical supervision. The study aimed to look at whether the ASCVD risk scores are different in those who developed ACS versus those who did not develop ACS.

Secondary objective

The secondary objective was to look at the impact of variables like hypertension, lipid profile parameters, family history of ACS and duration of diabetes on the development of ACS**.**

METHODS

Study design

This is an electronic medical record (EMR) based case-control study to understand the utility of the ASCVD risk calculator in predicting ACS as well as look at other risk factors that can predict ACS in T2DM.

Inclusion criteria

EMRs of people with T2DM who visited the outpatient clinic between 1st January 2016 to 31st December 2022 were analysed. Only those EMRs with complete details of age, sex, and body mass index, diagnosis of diabetes including timing of onset, family history of diabetes, blood pressure, lipid profile, renal and liver function tests (in those affected with ACS, the details need to be within 6 months prior to ACS) were included.

Exclusion criteria

EMRs of people with diabetes other than confirmed T2DM were excluded. Those with no documented visit within 6 months before developing an ACS, or subjects with chronic liver disease (transaminases more than 2 times the upper limit of normal or Child-Pugh class B and C), chronic kidney disease (eGFR<60 ml/min), known previous cardiovascular disease, cerebrovascular disease, or peripheral vascular disease, hyperhomocystinaemia, known familial hypercholesterolaemia, valvular heart disease or cardiac arrythmias, retroviral disease, pulmonary tuberculosis and severe chronic obstructive airway disease.

Endpoints

The current study looks at the comparison of ASCVD risk score calculated based on parameters prior to actual development of ACS in subjects with T2DM who developed ACS and age and sex-matched controls who did not develop ACS thereby testing the utility of ASCVD risk score in predicting ACS in subjects with T2DM on medical management. The individual impact of these factors on the development of ACS was also analysed.

Data parameters

Details of age (in years), sex, duration of diabetes (as available from patients' clinical records), family history of ACS (as recalled by the patient in first-degree relatives), blood pressure (measured and documented in EMR in mm hg), HbA1c and lipid profile (from the laboratory reports of the patient) were extracted from the EMRs for the cases and controls. The uniqueness of this population was the documented medical visit within the last 6 months before the occurrence of ACS with a qualified medical practitioner. As a result, most of the subjects were already on statins, taking antihypertensives and all were taking oral anti-diabetic medications ± insulin. ASCVD risk score for possible 10-year risk of ACS was calculated using the online ASCVD risk calculator.

Sample size

The EMRs of 4248 individuals with diabetes who visited a single outpatient practice (total of 15567 visits) between 1st January 2016 to 31st December 2022 were reviewed. Of these, 1226 EMRs matched the inclusion criteria, of which 207 people with T2DM had an ACS (documented fatal myocardial infarction, nonfatal myocardial infarction or unstable angina leading to hospitalisation and revascularisation from 1 January 2016 to 31 December 2022). The required sample size based on adverse cardiovascular event incidence in the CVD-REAL study (2.25 percent per patient year translating to 13.5 percent for six patient years) was found to be 180. With the sample size of 1226, the margin of error at a 95% confidence interval was found to be 2.1% and for a 99% confidence interval, it was 2.76%.[8] With a population size of 1226 and an ACS incidence rate of 16.88%, a sample size of 184 was found to be sufficient (5% margin of error, 95% confidence interval). At a sample size of 207, the margin of error with 95% confidence interval was 4.65%. The records of these 207 subjects who developed ACS were compared with 207 age and sex-matched controls. The controls were identified among the remaining 1019 EMRs. The ratio of cases to controls was 1:1.

Statistical analysis

Data are summarized as median and IQR. Wilcoxon rank-sum test was used for checking differences in continuous variables and Pearson's Chi-squared test for categorical data. The chi-square test was used to check differences in ordinal variables. To examine the relationship between these variables and the occurrence of acute coronary syndrome (ACS) beyond that explained by the ASCVD score, we conducted univariate and multivariate logistic regression analysis. The model utilised the forced entry method to assess the effect of variables other than the ASCVD score to predict the occurrence of ACS. The dependent variable is the occurrence of ACS.

Statistical data analysis is performed using JASP, version 0.16.4 (JASP Team [2022]) for MS Windows.

RESULTS

Of the 1226 EMRs of patients with T2DM under medical follow-up, 207 had ACS in the last 6 years. This gives an incidence rate of 16.88% over 6 years. This is more than the 10-year incidence predicted by the ASCVD risk calculator for 1226 people (13.85 ± 8.21) (p <0.05).

Furthermore, a case-control study conducted on 207 cases and 207 controls (adequate sample size estimated to be 184 assuming a population of 1226 and proportion of 16.88%) to understand the utility of ASCVD risk calculator in predicting atherosclerotic cardiovascular events among subjects with T2DM, as well as the impact of other variables like lipid profile, blood pressure, family history of diabetes, duration of diabetes, smoking status, and statin use on the development of ACS. The cases with ASCVD had a mean age of 56.8 ± 6.403 (Min: 34, Max: 65) while the controls without ASCVD had a mean age of 56.8 ± 6.430 (Min: 34, Max: 65). The mean ASCVD score was 14.565 ± 8.709 (Min: 1.5, Max: 38.3) for the cases and 13.114 ± 8.247 (Min: 1.4, Max: 45) for the controls, with no significant difference between the groups (p = 0.083). Breaking down the scores into Low (<5%), Borderline (5-7.5%), Intermediate (7.5-20%) and High (>20%), there were no significant differences between the two groups as well, with an equal and varied range of scores in both groups (Figure 1).

The cases had a median total cholesterol of 174 mg/dL (IQR: 148 to 197 mg/dL), while the controls had a median cholesterol level of 186 mg/dL (IQR: 175 to 191 mg/dL) (p = 0.007). The cases had a median systolic blood pressure of 140 mmHg (IQR: 124 to 154 mmHg), while the controls averaged 125 mmHg (IQR: 120 to 135 mmHg). Statin use was more prevalent in the cases, with 79% (n = 164) taking the drug compared to 63% (n = 130) in the control group (p <0.001). Family history of diabetes was notably different between the groups. While 34% (n = 141) of the entire cohort reported family history, it was significantly more common in cases (50%) than in controls (18%) with p <0.001. Overall, the median duration of diabetes was 8 years. Cases had a notably longer diabetes duration with a median of 12 years (IQR: 8 to 15 years) compared to controls with a median of 5 years (IQR: 3 to 9 years), which was statistically significant with p <0.001 (Table 1).

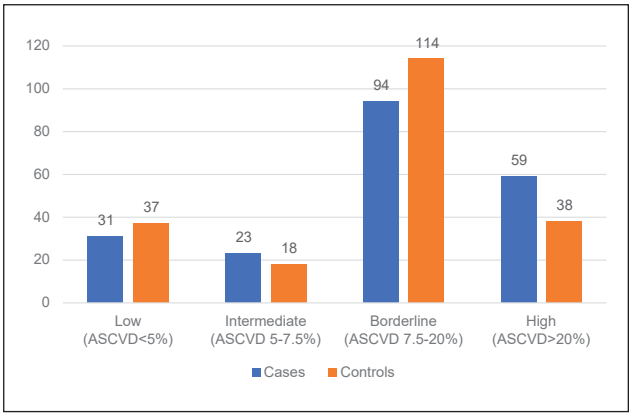

Figure 1. Number of subjects among cases and controls with ASCVD risk scores in the low, borderline, intermediate and high categories.

We found significant correlations between the ASCVD score and several health indicators (LDL, HDL, SBP, diastolic blood pressure, cholesterol, hypertension, smoking). Similarly, several factors were significantly and positively correlated with the occurrence of atherosclerotic cardiovascular events, including SBP, duration of diabetes, statin use, and family history.

In the univariate analysis, every unit increase in HbA1c was associated with 16% increased odds of the outcome, which bordered on statistical significance (OR: 1.16, 95% CI: 0.99, 1.36; p = 0.068). The multivariate analysis indicated a 17% increase in odds (OR: 1.17, 95% CI: 0.96, 1.44; p = 0.12). Every unit increase in systolic blood pressure was associated with a 3% and 4% increase in odds in the univariate (OR: 1.03, 95% CI: 1.02, 1.05; p <0.001) and multivariate (OR: 1.04, 95% CI: 1.03, 1.06; p <0.001) analyses, respectively. The use of statins was associated with a significant 126% increase in odds of the outcome in univariate analysis (OR: 2.26, 95% CI: 1.46, 3.52; p <0.001). A positive family history was strongly associated with the outcome. The univariate analysis demonstrated a 364% increase in odds (OR: 4.64, 95% CI: 2.99, 7.33; p <0.001), and the multivariate analysis showed a 470% increase (OR: 5.70, 95% CI: 3.41, 9.77; p <0.001). Every unit increase in the duration of diabetes was associated with a 17% increase in the univariate analysis (OR: 1.17, 95% CI: 1.13, 1.23; p <0.001) and a 19% increase in the multivariate analysis (OR: 1.19, 95% CI: 1.13, 1.25; p <0.001). The results of the regression analysis are summarized in Table 2.

The multivariate regression analysis showed that the probability of development of ACS was higher with elevated systolic blood pressure (OR: 1.04, 95% CI: 1.03, 1.06; p <0.001), positive family history (OR: 5.7, 95% CI: 3.41,9.77; p <0.001), and longer duration of diabetes (OR: 1.19, 95% CI: 1.13, 1.25; p <0.001). Raised HbA1c also showed a trend towards increased risk but did not reach statistical significance in the multivariate analysis (OR: 1.17, 95% CI: 0.96, 1.44; p = 0.12) though it was significant in the univariate analysis. Thus, the risk of ACS was increased by 4% per mm Hg increase in systolic pressure, 19 percent per year of increase in the duration of T2DM, and with a positive family history of ACS. One percent (1 %) change in HbA1c increased ACS risk by 17 % but did not reach statistical significance.

DISCUSSION

T2DM is a major risk factor for ASCVD which is the leading cause of mortality. With better screening programmes and access to care, a good number of patients with T2DM are in optimum medical management. Recent guidelines emphasize end-organ protection in addition to blood glucose control. Those who have established cardiovascular disease are subclassified separately and are recommended to be given cardioprotective treatment. However, a large section of people with diabetes has subclinical cardiovascular disease and are deserving candidates for cardioprotective treatment but get deprived. One way of

Table 1. Differences in the characteristics among diabetic subjects with (cases) and without (controls) ACS

Characteristic	Overall, N = 414*	Controls, N = 207*	Cases, N = 207*	p†
Sex				>0.9
Female	164 (40%)	82 (40%)	82 (40%)	
Male	250 (60%)	125 (60%)	125 (60%)	
Age in years	59 (54, 62)	59 (54, 62)	59 (54, 62)	>0.9
HbA1c in %	7.60 (7.00, 8.30)	7.60 (7.00, 8.10)	7.60 (7.00, 8.50)	0.6
LDL in mg/dl	86 (70, 96)	86 (78, 95)	84 (66, 100)	0.3
HDL in mg/dl	44 (38, 47)	44 (40, 48)	43 (35, 46)	0.10
Cholesterol in mg/dl	184 (160, 194)	186 (175, 191)	174 (148, 197)	0.007
Systolic BP in mmHg	133 (123, 145)	125 (120, 135)	140 (124, 154)	<0.001
Diastolic BP in mmHg	80 (78, 88)	82 (78, 88)	80 (80, 90)	0.052
Smoker	18 (4.3%)	9 (4.3%)	9 (4.3%)	>0.9
Hypertension	282 (68%)	132 (64%)	150 (72%)	0.058
Statin	294 (71%)	130 (63%)	164 (79%)	<0.001
Aspirin	132 (32%)	58 (28%)	74 (36%)	0.092
ASCVD	12 (7, 19)	12 (7, 18)	13 (7, 22)	0.092
Family history of diabetes	141 (34%)	37 (18%)	104 (50%)	<0.001
Duration of diabetes	8 (4, 12)	5 (3, 9)	12 (8, 15)	<0.001

*n (%); Median (IQR)
†Pearson's Chi-squared test; Wilcoxon rank sum test

Table 2. Results of univariate and multivariate logistic regression analyses for the variables with occurrence of ACS

Characteristic	Univariate				Multivariate		
	N	OR	95% CI	*p*-value	OR	95% CI	*p*
Sex	414						
Female		—	—		—	—	
Male		1.00	0.67, 1.48	>0.9	1.20	0.60, 2.44	0.6
Age	414	1.00	0.97, 1.03	>0.9	1.01	0.95, 1.08	0.7
HbA1c	414	1.16	0.99, 1.36	0.068	1.17	0.96, 1.44	0.12
LDL	414	1.00	0.99, 1.01	0.5			
HDL	414	1.00	0.97, 1.02	0.7			
Cholesterol	414	1.00	0.99, 1.00	0.5	1.00	0.99, 1.01	0.7
Systolic BP	414	1.03	1.02, 1.05	<0.001	1.04	1.03, 1.06	<0.001
Diastolic BP	414	1.02	0.99, 1.04	0.2	0.99	0.96, 1.02	0.5
Smoker	414	1.00	0.38, 2.61	>0.9			
Hypertension	414	1.50	0.99, 2.27	0.058			
Statin	414	2.26	1.46, 3.52	<0.001			
Aspirin	414	1.43	0.94, 2.17	0.092			
ASCVD	414	1.02	1.00, 1.04	0.083	0.97	0.92, 1.03	0.4
Family history of diabetes	414	4.64	2.99, 7.33	<0.001	5.70	3.41, 9.77	<0.001
Duration of diabetes	414	1.17	1.13, 1.23	<0.001	1.19	1.13, 1.25	<0.001

OR = Odds Ratio, CI = Confidence Interval

identifying those at risk is the use of the ASCVD risk score. However, its predictive value in patients on treatment is uncertain and it is important to identify those at risk.[3,4]

Asian Indians are an ethnic group with a higher risk of developing IHD and ACS. This can be one of the limitations of applying the ASCVD risk score in Asian Indians. The present study confirms this by noting a higher incidence of ACS (in 6 years only) than predicted by the ASCVD risk score (which predicts a 10-year probability).[5,6]

The risk engines have always underestimated the value of family history which is one of the most important determinants of ACS and ASCVD. This study highlights the relevance of family history. Those with a negative family history were 30% less likely to develop ACS. This has been seen even in previous studies looking at the relevance of family history in ACS. It also highlights the relevance of raised SBP in the development of ACS. This calls for more proactive and aggressive control of blood pressure especially in those who are vulnerable.[7,9,10]

It is a known fact that the duration of diabetes is relevant in the development of diabetic complications and this study highlights its relevance in the development of ACS. This also substantiates the fact that those who get T2DM early should be more aggressively treated to target.[11]

Participants who are on statins have odds of having an ACS that is 2.26 times higher than those without statins, indicating that these are higher-risk individuals. It is proven beyond doubt that statin use is the mainstay of protection against ASCVD. Also, the current population is under medical supervision. This reflects more baseline dyslipidaemia in the cases and favourable baseline lipid profile in controls since the LDL levels are not different in the two groups. Because the LDL and total cholesterol levels were not different in the two groups but the number using statins was more in the ACS group implied that baseline dyslipidaemia for a duration pre-existed in this population which undermined the fact that early treatment of dyslipidaemia may also have a legacy effect in the prevention of ASCVD.

The study represents the presence of unaddressed residual risk in a population managed as per guidelines. It also stresses the felt need for the usage of methods other than the ASCVD risk calculator which is a decade old now. This calls for better risk stratification using more objective tools like biomarkers (hsCRP, NT-pro BNP) or radiological non-invasive modalities (e.g., coronary calcium scoring, carotid intima-media thickness, etc.) in a vulnerable population (the authors believe Southeast Asians fall in this group), especially with T2DM and positive family history of ASCVD. This is extremely important because the guidelines of T2DM care are now based on cardiac risk stratification and a large population base who are at risk of heart disease may be deprived of cardioprotective medications.[12-14]

The study also highlights the factors to be looked at in those who are under supervised care for T2DM as per current standards and may help clinicians identify people who need more attention notably those with positive family history, longer duration of diabetes, and uncontrolled SBP.

Limitations of the study

This being an EMR-based single centre and retrospective study, the observations need to be verified in a prospective study. Also, the subjects may not represent the general population since they were already diagnosed and in follow-up with their physicians. Most of the people in the study are also residing within a specific geographic area, and so, the conclusions may not be generalizable. The study excluded patients with chronic liver and kidney diseases, known previous cardiovascular and cerebrovascular diseases, and other conditions, which may also affect the generalizability of the findings. The study did not consider

the effect of lifestyle factors, such as diet and exercise, as well as compliance with medications which may affect the occurrence of ACS.

CONCLUSION

The study highlights the presence of residual risk in a population treated as per standards of care. Most of the risk engines including the ASCVD risk scoring are well-validated and continue to remain relevant but still have limitations. One of these is the lack of validation of these risk engines in patients already on treatment. The study emphasizes the importance of family history and longer duration of diabetes as non-modifiable risk factors needing additional vigilance and consideration beyond risk engines and blood pressure control as a modifiable risk factor that stands out despite being part of the risk engines too. It also calls for exploring other options for early diagnosis of ASCVD for better risk stratification and optimising medical management.

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

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

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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ORIGINAL ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

Prevailing Food Intake, Physical Activity and Health Beliefs in a Rural Agricultural Community in the Philippines: Factors to Consider Prior to a Diabetes Prevention Program

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Abstract

Objective. A diabetes prevention program is being proposed in the rural agricultural town of San Juan, Batangas, Philippines. This study aims to determine the prevailing level of food intake, physical activity, and health beliefs prior to any intervention.

Methodology. Adults were recruited via random sampling with proportional allocation. Interviews were done to determine food intake and physical activity. Small group discussions were held to determine prevailing health beliefs.

Results. The average energy intake (1,547 kcal/d) is only 72% of the recommended values for Filipinos. Only 12% of the respondents achieved the recommended energy intake. Carbohydrates comprise a large part (71%) of calorie intake. A majority (91%) already have moderate to high levels of physical activity. There are prevailing health beliefs that need to be considered when dietary modifications and physical activity interventions are to be done.

Conclusion. Internationally recommended diabetes prevention interventions such as reducing calorie intake and increasing physical activity may not be directly applicable here. We recommend that the features of a diabetes prevention program for this locale must include the following: 1) introduction of affordable plant sources of proteins; 2) decreasing the proportion of rice as a source of carbohydrates in the diet; 3) maintaining the level of physical activity; and 4) being sensitive to the prevailing health beliefs.

Key words: beliefs, diabetes prevention, diet, lifestyle, prediabetes, rural community

INTRODUCTION

In the Philippines, the national prevalence of diabetes has increased from 4.6% in 2003 to 6.0% in 2008.[1,2] Given that the treatment of diabetes mellitus carries a high financial burden, interventions that will prevent its development are valuable in resource-poor communities and would have significant public health effects.

A large, multiphase study on diabetes is being conducted in San Juan, Batangas, Philippines. Phase I dealt with determining the knowledge, attitudes, and practices of patients with diabetes. It was found that the mean score on knowledge was just 43%, reflecting a gap in diabetes knowledge, and only 1% believed that it is a serious disease.[3] Diabetes, pre-diabetes, and metabolic syndrome were then identified to be important public health concerns during Phase II.[4] Prevailing socio-economic realities led to volunteer bias and an overestimation of the prevalence values, which demonstrated how socio-economic realities influence the conduct of scientific studies in the rural community. In Phase III, a diabetes self-management education program was implemented which resulted in lower levels of hemoglobin A1C (HbA1c) and a larger proportion of participants achieved HbA1c <7% among those who received the intervention.[5] In addition, there was a change in health behavior, as there were more diabetics in the intervention group who now perform regular foot examinations.

eISSN 2308-118x (Online)
Printed in the Philippines

Received: April 13, 2023. Accepted: July 19, 2023.
Published online first: January 23, 2024.
https://doi.org/10.15605/jafes.039.01.11

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The next phase is the implementation of a locally acceptable diabetes prevention program in the same community. The American Diabetes Association Standards of Medical Care states that lowering calorie intake and increasing physical activity help in diabetes prevention.[6] However, to come up with an effective diabetes prevention program, it is paramount to determine the level of food intake and physical activity prevailing in the community and if the recommendations of a foreign organization are applicable to our local setting.

OBJECTIVES

1. To determine the prevailing level of food intake of the adult population in San Juan, Batangas;
2. To determine the prevailing level of physical activity of the adult population in San Juan, Batangas;
3. To determine the prevailing health beliefs associated with food intake and physical activity among the adult population in San Juan, Batangas.

METHODOLOGY

Study design

Cross-sectional study

Study site

The study site is the municipality of San Juan, Batangas in the Philippines. It is an agricultural coastal town which is 120 kilometers away from the country's capital, Manila. This is the chosen site as there is already an existing partnership between the municipal government of San Juan and the University of the Philippines Manila.

Based on the then National Statistics Office (now Philippine Statistics Authority) Census of Population and Housing of 2010, San Juan had a total population of 94,232. It had an adult (20 years and above) population of 38,187, of which 23,498 (61.5%) were 20-39 years old, 10,964 (28.7%) were 40-59 years old, and 3,725 (9.7%) were 60 years old and above. There were 19,312 (50.6%) adult males and 18,875 (49.4%) adult females.[7]

The town is composed of 43 villages (barangays) divided into 12 geographical clusters. Table 1 shows the population per village cluster and its proportion to the total population.

Participants

In 2013, the authors recruited the participants of this study via stratified random sampling of community residents. The strata for sampling were the village (barangay) cluster, age, and sex. There were 12 clusters and one village (barangay) was chosen per cluster. A proportionate number of participants were taken from each stratum. Hence, villages with larger populations were represented by a larger number of participants.

The following formula was used for the computation of sample size:

$$n = \frac{N}{(1 + Ne^2)};$$

where adult population size (N) = 38,187; margin of error (e) = 0.10

Based on this formula, the minimum sample size is 99.7, rounded off to 100 participants. To allow for a 20% drop out rate, we planned to recruit 120 participants.

Ethical considerations

The research design and objectives of this study were first communicated to the local health authorities. Written informed consent from all participants was secured. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines of the Research Implementation and Development Office (RIDO) of the College of Medicine and Research Ethics Board of the University of the Philippines Manila (UPMREB) [Study Code: GCS-IM-2010-001 (R-001TE)].

Table 1. San Juan, Batangas village (barangay) clusters and population

Cluster	Villages (Barangays)	Cluster Population	Proportion of the Town's Population
1	Poblacion, Maraykit, **Calicanto**, Janao-janao, Sico 1.0, Sico 2.0, Palahanan 1.0, Palahanan 2.0	16,928	18.51%
2	Pinagbayanan, Poctol, **Balagbag**, Catmon	6,713	7.34%
3	**Tipaz**, Calit-calit, Lipahan, Paling-owak	10,698	11.70%
4	Escribano, **Libato**, Muzon	7,873	8.61%
5	**Talahiban 1.0**, Talahiban 2.0, Mabalanoy	6,710	7.34%
6	Buhaynasapa, Ticalan, **Sampiro**, Sapangan	10,482	11.46%
7	Abung, **Subukin**, Calubcub 1.0, Calubcub 2.0	8,153	8.92%
8	**Putingbuhabngin**, Pulanbato, Quipot	7,251	7.93%
9	Nagsaulay, **Bataan**, Coloconto	4,225	4.62%
10	**Imelda**, Barualte	2,213	2.42%
11	**Laiya Ibabao**, Bulsa	5,185	5.67%
12	Laiya Aplaya, **Hugom**	4,999	5.47%
Total		91,430	100.00%

Note: Highlighted and underlined villages were the selected villages from where participants were recruited.

Data collection and analyses

Information on dietary intake was obtained through a 2-day 24-hour food recall; participants were visited twice and asked about the food items and beverages they consumed for the duration. The equivalent calorie and macronutrient content were then determined using the Food Composition Tables of the Philippine - Food and Nutrition Research Institute (FNRI).[8] Results of the dietary intake survey were then compared to the recommended intake as stated in the Recommended Energy and Nutrient Intakes (RENI) of FNRI.[9] The conduct of the 24-hour food recall was performed by endocrinologists and three registered nutritionist-dietitians in our team, while the determination of equivalent calorie and macronutrient content of the data collected from the food recall was done by three registered nutritionist-dieticians in the team.

Physical activity was assessed by making participants recall the duration and type of physical activity they performed in the past 7 days. The International Physical Activity Questionnaire,[10] an instrument designed for population surveillance of physical activity among adults, was administered. The amount of physical activity can be computed by weighing each type of activity by its energy requirements defined as metabolic equivalents (METs).[11] The average energy consumption as a continuous variable was estimated in MET-minutes per week. The level of physical activity was also expressed as categorical variables and classified into three categories: low (<600 MET-min/wk), moderate (600-3000 MET-min/wk) and high (>3,000 MET-min/wk).

All recruited community residents participated in small group discussions held in their respective village health centers. The number of participants in a group ranged from 6 to 21 participants. Discussions were conducted in the Filipino language. These were facilitated by an endocrinologist assisted by a community organizer who was more familiar with the local dialect. An open-ended question was asked to start the discussion: *Here in your village, do you have any specific beliefs regarding food intake, such as what food items should be consumed, and which should not be consumed for whatever reason?*

A similar question was asked when the discussion moved to physical activity. Participants were reminded that there are no wrong answers. Responses were then listed. If the belief was mentioned by at least one participant, it was included in the list of gathered health beliefs. The responses were then arranged according to similar themes.

Statistical analysis

Continuous variables were presented as mean or median with standard deviation and interquartile ranges. Categorical variables were presented as percentages.

To determine whether the variables follow a normal distribution, the Kolmogorov-Smirnov test for normality was performed. A p-value greater than 0.05 indicates that there is no sufficient evidence to say that the normality assumption is violated.

To determine any significant differences between males and females (two groups being compared), an independent samples t-test was performed for variables with a normal distribution. Student's independent samples t-test was done if the variables had equal variances. Welch's independent samples t-test was done if the variables had unequal variances. Levene's test, which determines whether variances are equal, was done to determine which of the two – Student's or Welch's independent samples t-test – is appropriate to perform. For variables that do not have a normal distribution, Mann-Whitney's test was performed instead.

To determine any significant differences between the age groups 20-39, 40-59, and 60+ y/o age groups (more than two groups being compared), a one-way analysis of variance (ANOVA) was performed for variables with a normal distribution. To check for normality, we used the Kolmogorov-Smirnov test. To determine if variances are equal, Levene's test was done. If normally distributed and with equal variances, Fisher's one-way ANOVA (with Tukey's as a post hoc test) was used. If normally distributed and with unequal variances, Welch's one-way ANOVA (with Games-Howell as a post hoc test) was used. If not normally distributed, the Kruskal-Wallis test was done regardless if the variances are equal or not (with the Dwass-Steel-Crutchlow-Fligner pairwise comparison test as post hoc test).

Computations were performed using Jamovi Version 2.3. (The jamovi project (2022). *jamovi*. (Version 2.3) [Computer Software]. Retrieved from https://www.jamovi.org. Jamovi is based on R. R Core Team (2021). *R: A Language and environment for statistical computing*. (Version 4.1) [Computer software]. Retrieved from https://cran.r-project.org. (R packages retrieved from MRAN snapshot 2022-01-01).

RESULTS

A total of 139 adults (70 males and 69 females) were recruited for this study, more than the required minimum sample size of 100. The overall mean age was 42 years, with the youngest at age 21 and the oldest at age 79. Fifty-one percent (51%) are in the 20- to 39-year-old age group, 31% in the 40- to 59-year-old age group, and 18% in the 60-year-old and above age group. The mean body mass index (BMI) is 22.68 kg/m^2 (mean weight = 57.49 kg; mean height = 1.59 m).

Participants were recruited from 12 village clusters *(barangays)*, with the number recruited per village being in proportion to the cluster's population (Table 2).

Table 3 shows the mean food intake expressed as total calories and grams of macronutrients. Likewise, calorie intake expressed as a proportion of the RENI is stated, and the proportion of participants achieving their corresponding RENI. Overall, the mean energy intake is only 1,547 kcal/d. Carbohydrates comprised 71% of the total energy intake, while proteins and fats accounted for 14% and 13%, respectively. The mean energy intake is only 72% of RENI and only 12% of the participants achieved recommended energy intake levels.

As presented in Figure 1 and Table 3, males had a higher energy intake (1,741 vs. 1,351 kcal/d, p <0.001), higher carbohydrate intake (305 vs. 241 g/d, p <0.001), and higher protein intake (64 vs 46 g/d, p = 0.001) compared with females. Fat intake and energy intake as % of RENI were similar for males and females. Comparing age groups, the 20-39-year-old age group recorded a higher energy intake than the 60-year-old and above age group (1,664 vs. 1,308 kcal/d, p = 0.005).

The physical activity of the study population in mean metabolic equivalents is presented in Figure 2 and Table 4. The mean level of physical activity is 4,829 MET-min/wk, which corresponds to a high level of physical activity. The median is 2,844 MET-min/wk. High (>3,000 MET/min/wk), moderate (600-3,000 MET-min/wk), and low (<600 MET-min/wk) levels of physical activity were reported by 47%, 44%, and 9% of participants, respectively. Males had a higher level of physical activity than females (6,557 vs. 3,076 MET-min/ wk, p = 0.003). There are no differences in the level of physical activity across the various age groups (Figure 2).

With regards to the beliefs on food intake, the responses were categorized into the following themes: (a) food and their nutritional value; (b) food and specific medical

Table 2. Proportional allocation of recruited participants stratified according to sex, age, and village (barangay) cluster

Cluster and Barangay	20-39 years old		40-59 years old		60 years old and above		Total	Proportion
	Male	Female	Male	Female	Male	Female		
1 - Calicanto	6	6	3	3	2	1	21	15.11%
2 - Balagbag	3	3	2	2	1	1	12	8.63%
3 - Tipaz	4	4	2	2	1	1	14	10.07%
4 - Libato	3	3	2	2	1	1	12	8.63%
5 - Talahiban 1.0	3	3	2	2	1	1	12	8.63%
6 - Sampiro	4	4	2	2	1	1	14	10.07%
7 - Subukin	3	3	2	2	1	1	12	8.63%
8 - Putingbuhangin	3	3	2	2	1	1	12	8.63%
9 - Bataan	2	2	1	1	1	1	8	5.76%
10 - Imelda	1	1	1	1	1	1	6	4.32%
11 - Laiya Ibabao	2	2	1	1	1	1	8	5.76%
12 - Hugom	2	2	1	1	1	1	8	5.76%
							139	100.00%
Total for the age group	72		42		25			
Proportion	51.80%		30.22%		17.99%			

Table 3. Nutritional data from food intake of adult residents in the rural community of San Juan, Batangas

Category	N	Carbohydrates (g) Mean (SD), % of total calories	Protein (g), Mean (SD), % of total calories	Fats (g), Mean (SD), % of total calories	Energy (kcal), Mean (SD)	% of RENI, Mean (SD)	Partcipants above RENI, n (%)
Overall	139	273 (93), 71%	55 (36), 14%	23 (18), 13%	1547, (558)	72%, (25)	16 (12%)
Sex							
Males	70	305 (105), 70%	64 (46), 15%	24 (22), 12%	1,741 (649)	69% (25)	7 (10%)
Females	69	241 (65), 71%	46 (20), 14%	23 (13), 15%	1,351 (358)	76% (25)	9 (13%)
p (males vs females)		**<0.001***	**0.001***	0.312	**<0.001***	0.072	
Statistical test performed		Welch's independent t-test	Mann-Whitney test	Mann-Whitney test	Welch's independent t-test	Student's independent t-test	
Age group (y/o)							
20-39	71	291 (99), 70%	55 (24), 13%	26 (20), 14%	1,661 (630)	72% (27)	9 (13%)
40-59	43	259 (84), 69%	60 (56), 16%	23 (18), 14%	1,493 (461)	68% (22)	2 (5%)
60+	25	247 (82), 76%	45 (18), 14%	15 (10), 10%	1,308 (399)	81% (26)	5 (20%)
p (20-39 vs 40-59 vs 60+y/o)		0.057	0.15	**0.029***	**0.007***	0.133	
Statistical test performed		Fisher's one-way ANOVA	Kruskall-Wallis test	Kruskall-Wallis test	Welch's one-way ANOVA	Fisher's one-way ANOVA	
p (20-39 vs 40-59 y/o)		N/A	N/A	0.59	0.221	N/A	
P (20-39 vs 60+ y/o)		N/A	N/A	**0.023***	**0.005***	N/A	
p (40-59 vs 60+ y/o)		N/A	N/A	0.192	0.199	N/A	
Post hoc statistical test performed		N/A	N/A	Dwass-Steel-Crutchlow-Fligner pairwise comparison	Games-Howell test	N/A	

*p <0.05, statistically significant

conditions; (c) food intake among pregnant women; (d) food intake among women who have just given birth; (e) food intake during the wake of a deceased relative; and (f) food intake on special occasions. These beliefs are arranged and categorized in Table 5, whether they agree or not with the generally accepted health recommendations.

Health beliefs on physical activity and exercise (Table 6) were categorized into (a) health benefits of exercise; (b) physical activity during the Lenten Season; (c) physical activity among women; and (d) physical activity during the wake of a deceased relative; and (e) exercise and work.

DISCUSSION

Elements of diabetes intervention programs include a reduction in calorie intake and an increase in the level of physical activity.[6] The results of this study show that the calorie equivalent of the average food intake in this community is suboptimal. This suggests that decreasing the total calorie intake is an inappropriate intervention since it could worsen malnutrition.

Carbohydrates contribute a large part of the calorie intake, larger than the usual 50%-60% that is generally recommended. This is not surprising since locally available carbohydrate-rich food, mainly rice, is the affordable one. Participants find meat and fruits expensive; hence, these do not form part of their usual diet. The ideal intervention to be conducted should focus on not lowering the total calorie intake but decreasing the proportion of carbohydrates and increasing that of proteins. If meat is not affordable, consumption of eggs and plant-derived proteins such as beans and tofu can be emphasized as alternatives.

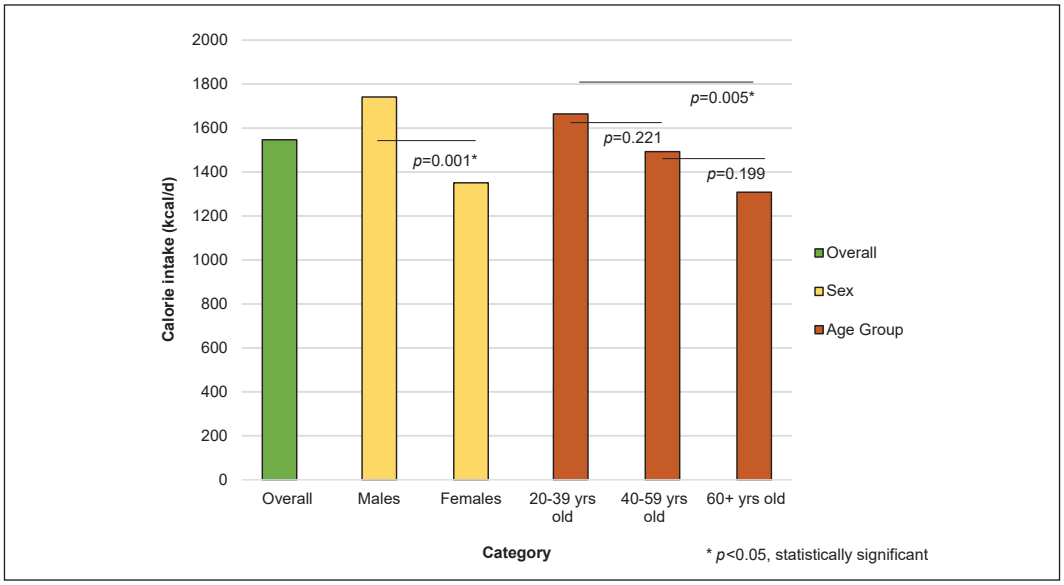

Figure 1. Calorie equivalent of average daily food intake of adult residents in the rural community of San Juan, Batangas.

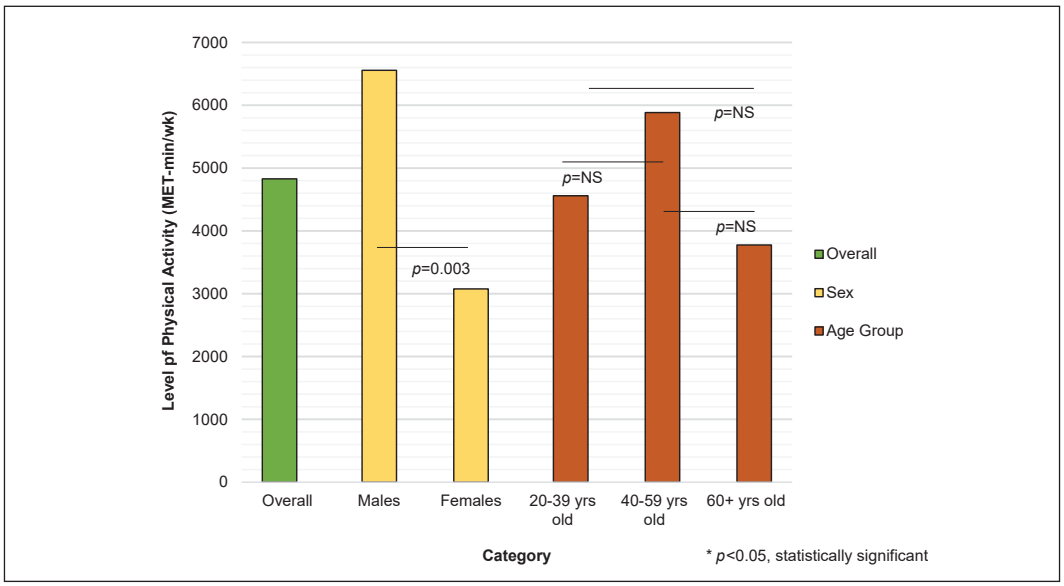

Figure 2. Level of physical activity of adult residents in the rural community of San Juan, Batangas.

Table 4. Level of physical activity of adult residents in the rural community of San Juan, Batangas

Category	N	Mean Level of Physical Activity (MET-min/wk)	Number (%) of participants within a category of the Level of Physical Activity		
			High (>3,000 MET-min/wk)	Moderate (600-3,000 MET-min/wk)	Low (<600 MET-min/wk)
Overall	139	4,829	65 (47%)	61 (44%)	13 (9%)
Sex					
Males	70	6,557	43 (61%)	22 (31%)	5 (7%)
Females	69	3,076	22 (32%)	39 (57%)	8 (12%)
p value (males vs females)		**0.003***			
Statistical test performed		Mann-Whitney test			
Age group (y/o)					
20-39	71	4,560	32 (45%)	32 (45%)	7 (10%)
40-59	43	5,885	21 (49%)	20 (47%)	2 (5%)
60+	25	3,776	12 (48%)	9 (36%)	4 (16%)
p (20-39 vs 40-59 vs 60+ y/o)		0.545			
Statistical test performed		Kruskall-Wallis test			

*p <0.05, statistically significant

Men had a higher energy intake than women. This is understandable since men engage in more laborious activities. This is corroborated by our finding that men do engage in a higher level of physical activity than women. Energy intake was likewise higher in the 20-39-year-old age group compared to those aged 60 years and above since the younger age group belongs to the labor force. Almost half (47%) have a high level while an almost similar number (44%) have a moderate level of physical activity. It can be inferred that the locale already has a physically active population. Improvement in physical activity is no longer needed but should just be maintained.

Part of a healthy diet is the consumption of fruits and vegetables. However, probing into their health beliefs (Table 5), it has been shown that there are social situations wherein consumption of vegetables is not acceptable. For example, pregnant women are advised not to eat eggplant to prevent their baby from getting dark-skinned. Also, when the family is observing a wake in honor of a deceased relative, they believe that eating vegetables from plants that crawl such as vines (i.e., chayote, gourd, squash, bitter gourd), shed leaves and blossoms (i.e., banana and horseradish "malunggay", or exude sap (i.e., papaya, okra, chayote, star apple) bring bad luck to the family.

It can also be seen in their beliefs (Table 5) that consumption of carbohydrate-rich foods is encouraged depending on the social context. Sticky rice cakes are encouraged to be partaken of during local festivities and All Souls' Day. Likewise, rice cakes are given by a newly wedded couple to their principal sponsors since they expect to be given money in return. Also, married couples are encouraged to eat sticky rice and noodles, so they stick together and have a long-married life. This habit of eating rice cakes is a potential obstacle if limiting carbohydrate intake is needed for diabetes prevention.

There are also health beliefs that discourage physical activity in certain situations (Table 6). It is believed that women should not engage in strenuous activity during their menstrual period. Also, physical activities are frowned upon during religious events such as during Holy Week.

These social norms should be taken into consideration when recommending increasing vegetable consumption, lowering carbohydrate intake, and increasing physical activity. Implementers of health programs must be sensitive to these local health beliefs.

We recommend that the features of a diabetes prevention program for this particular locale must include the following: 1) introduction of affordable plant sources of proteins; 2) decrease the proportion of rice as a source of carbohydrates in the diet; 3) maintain the level of physical activity; and 4) being sensitive to the prevailing health beliefs.

The strength of this study is the random selection of participants using proportional allocation. The data gathered could be considered truly representative of the adult residents of this town. Determination of prevailing food intake and level of physical activity before the implementation of any health intervention shows that recommendations from other countries may not always be applicable in local settings. Additionally, knowing the prevailing health beliefs before the implementation of any health program is an expression of respect toward the locale's social norms. To have a successful program, any health intervention must not contradict prevailing beliefs.

Making use of food recall and physical activity questionnaires may be a limitation of this study since the actual food intake and level of physical activity were not measured. However, it can be argued that the use of questionnaires is considered acceptable in public health settings. The IPAQ is valid and reliable in various geographic settings.[11] The performance of more accurate methods like food measurements in their homes might be intrusive. Likewise, actometers may be accurate tools for measuring physical activity but they are too costly.

CONCLUSION

The average calorie intake in this rural agricultural town is suboptimal. The energy intake is only 72% of the recommended value for Filipinos. Only 12% of the respondents achieve the recommended energy intake.

Table 5. Health beliefs of adult residents regarding food intake

In Agreement with Generally Accepted Health Recommendations	Not in Agreement with Generally Accepted Health Recommendations
Food and their nutritional value	
1. Vegetables are nutritious. 2. Fish is rich in protein. 3. Meat is high in cholesterol. 4. Fish and vegetables are healthy to eat. 5. We should eat vegetables as these are both delicious and nutritious.	
Food and specific medical conditions	
1. Pork is bad for people with high blood pressure. 2. Persons with diabetes should limit their food intake. 3. Soft drink consumption can cause diabetes. 4. Excessive rice intake can cause diabetes. 5. Sweet foods are bad for people with diabetes. 6. Bitter gourd (locally known as ampalaya, *(Momordica charantia)* is good for people with diabetes. 7. Beans are bad for people with painful joints. 8. Pork and fatty foods are bad for the elderly.	1. Salty junk foods can cause urinary tract infections
Food intake among pregnant women	
1. Drinking milk is good for pregnant women. 2. Salty foods such as salted shrimp paste (“bagoong”) are bad for pregnant women.	1. Pregnant women should not eat eggplant to avoid their baby from being dark-skinned. 2. While pregnant and after giving birth, women should not eat fish with red meat to avoid having postpartum bleeding. 3. Eat the “makabuhay” plant *(Tinospora rumphii)* as a means of birth control to avoid getting pregnant.
Food intake among women who have just given birth	
1. Deer meat is good for these women so they can regain their strength faster. 2. Mushrooms are good for these women so they will not experience “binat” (loosely translated as “relapse”). 3. “Malunggay” (horse radish, *Moringa oleifera*) is good for enhancing breastmilk production. 4. Postpartum mothers should take a bath using water in which sour leaves (i.e. tamarind *Tamarindus indica*, locally known as “sampalok”) have been boiled. 5. For postpartum mothers, it is good to eat banana heart before taking their first bath to avoid the uterus from prolapsing. 6. Eating seafood is healthy for postpartum mothers.	1. Carabao meat should be avoided so as not to experience “binat” (loosely translated as “relapse.”)
Food intake during the wake of a deceased relative	
1. Fish should be eaten when there is a deceased family member. 2. Avoid sticky foods such as rice cakes when there is a deceased family member.	1. Do not eat vegetables that grow on crawling vines such as squash, chayote or gourd to avoid death from crawling or spreading in the family. 2. Do not eat vegetables that shed, like banana heart blossoms and “malunggay” leaves, to avoid another death in the family. 3. Avoid vegetables that come from plants which exude much sap such as papaya, okra or star apple lest the family members will not stop crying.
Food and special occasions	
1. People who have their foreheads applied with ash during Ash Wednesday should not eat food that has been prepared with blood (“dinuguan” or pork blood stew.).	1. During All Souls’ Day, partake of sticky rice cakes. 2. Newlyweds are encouraged to give sticky rice cakes to their wedding sponsors since it is expected that they will be given money in return. 3. The bride and groom should eat cake and noodles so their life together would be sweet and long. 4. Sticky rice cakes should be partaken of during feasts.

Table 6. Health beliefs of adult residents regarding physical activity.

In Agreement with Generally Accepted Health Recommendations	Not in Agreement with Generally Accepted Health Recommendations
Health benefits of exercise	
1. Exercise is good to strengthen the body and to control blood pressure. 2. It is not good if we do not sweat or perspire, thus we should exercise.	
Physical activity during the Lenten Season	
	1. Do not use a “palo-palo”, a wooden implement, when doing the laundry during Holy Week. 2. It is prohibited to take a bath or to swim during Holy Week. 3. We should refrain from working during Holy Week.
Physical activity among women	
1. For women, it is acceptable for a married woman to engage in ballroom dancing with another man other than his husband.	1. For men, married women will be frowned upon when engaged in ballroom dancing with men other than their husbands. 2. Women who have just given birth should not do the laundry nor clean the house to avoid experiencing “binat” (loosely translated as “relapse”). 3. Women who currently have their menses should avoid carrying heavy objects.
Physical activity during the wake of a deceased relative	
	1. It is prohibited to sweep the floors during the wake of a deceased relative.
Exercise and work	
1. Farming and tending fields is already a form of exercise.	1. We can only exercise when we are on vacation or holiday. 2. Exercise is only for those who do not work. 3. We do not have time for exercise. Working and walking are already exercise for us.

Carbohydrates comprise a large part (71%) of the calorie intake. A large majority (91%) already have moderate to high levels of physical activity. There are prevailing health beliefs that need to be considered when dietary modifications and physical activity interventions are to be done.

Internationally recommended diabetes prevention interventions such as reducing calorie intake and increasing physical activity may not be directly applicable in the studied rural agricultural community. We recommend that the features of a diabetes prevention program for this particular locale must include the following: 1) introduction of affordable plant sources of proteins; 2) decrease the proportion of rice as a source of carbohydrates in the diet; 3) maintain the level of physical activity; and 4) being sensitive to the prevailing health beliefs.

Acknowledgments
The authors are grateful to the local government of San Juan, Batangas led by Mayor Rodolfo Manalo, together with municipal health officer Dr. Nestor Alidio; the midwives and village health workers; and to the residents of San Juan for treating us with Filipino hospitality as they welcomed us into their homes and villages. Special thanks to Professor Maria Rosario Araneta, PhD of the Department of Family Medicine and Public Health of the University of California San Diego, for her valuable comments and suggestions when she reviewed the paper. We also acknowledge the statistical analysis performed by statistician Associate Professor Kevin Carl Santos, PhD.

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

CRediT Author Statement
MAS: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **EPP:** Conceptualization, Methodology, Resources, Writing – review and editing, Supervision, Project administration; Funding acquisition; **EC:** Investigation, Writing – review and editing; **PP:** Investigation, Writing – review and editing; **MTCC:** Investigation, Writing – review and editing; **FLLA:** Conceptualization, Methodology, Writing – review and editing, Supervision; **EP:** Conceptualization, Methodology, Writing – review and editing, Supervision; **NJ:** Conceptualization, Methodology, Writing – review and editing, Supervision; **CJ:** Conceptualization, Methodology, Writing – review and editing, Supervision

Author Disclosure
Dr. Pacheco is the Editor-in-Chief of JAFES. Dr. Jimeno is the Vice Editor-in-Chief of JAFES.

Funding Source
This study was funded by the Philippine Council for Health Research and Development (PCHRD) of the Department of Science and Technology (DOST) of the Philippines.

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ORIGINAL ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

Age and Sex-related Chromogranin A Gene Polymorphisms and its Association with Metabolic Syndrome Components

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Abstract

Introduction. The purpose of this study was to determine the possible differences in genetic polymorphisms and serum levels of chromogranin A (CgA), according to age and sex, in subjects with and without metabolic syndrome (MetS).

Methodology. The genotyping and serum level of CgA and biochemical parameters were measured by the T-ARMS-PCR and PCR-RFLP and ELISA and spectrophotometer methods, respectively.

Results. A comparison of males with and without MetS showed significantly lower high-density lipoprotein-cholesterol (HDL-C) levels than those of females.

At ages 30-70 years, both sexes showed significant differences in triglycerides (TG), fasting blood sugar (FBS), CgA levels and waist circumference (WC) when compared to the two groups. Both sexes with MetS indicated significant differences in systolic blood pressure (SBP) at ages 40-70 years, while at ages 40-59 years, there was a significant difference in HDL-C level in males.

There was a significant correlation between serum levels of FBS, TG, SBP and WC (in both sexes), and CgA in subjects with MetS. Significant correlation was found between HDL-C level and diastolic blood pressure (DBP), and CgA level in males and females, respectively. CgA genotype frequency (T-415C and C+87T polymorphisms) showed no significant differences between males and females with and without MetS, while there was only a significant difference in frequency of the genotypes T-415C when compared to males with and without MetS.

Conclusion. The CgA appears to be strongly associated with MetS components in both sexes. Variation in CgA gene expression may affect the T–415C polymorphism in males. This may mean that the structure of CgA genetics differs in different ethnic groups. Differences in the serum level and expression of CgA gene may show valuable study results that it may be expected a relationship between these variables and the MetS.

Key words: Age, Sex, Chromogranin A, genotype, metabolic syndrome

INTRODUCTION

Metabolic syndrome (MetS) is known as a significant factor of cardiovascular disease in the general population.[1] Many studies have indicated the relationship between MetS and coronary artery diseases in different ethnic groups, sex, age and countries.[2-7] Some findings have shown that MetS prevalence changes between 10 to 84% worldwide,[8] while some other studies revealed the MetS prevalence between 8 to 24% and 7 to 46.5% among males and females worldwide, respectively.[9,10] MetS is increasing from 20% to 30% among males and females in Europe[11,12] and increasing in Asian countries.[13] It has been reported that subjects with MetS are more at risk of developing diabetes and coronary heart disease.[14] Metabolic syndrome is a complicated disease.

Banks and Helle reported an adrenal medulla protein release in September 1965.[15] Blaschko et al.,[16] showed soluble protein release and called it chromogranin in July 1967. In September 1967, they named the protein chromogranin A (CgA) as the major component of these proteins.[17]

Chromogranin A (CgA) is a 48-52 kDa soluble acid glycoprotein that is widely secreted in the secretory vesicles

eISSN 2308-118x (Online)
Printed in the Philippines

Received: May 25, 2023. Accepted: July 22, 2023.
Published online first: January 9, 2024.
https://doi.org/10.15605/jafes.039.01.09

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of endocrine, neuronal and neuroendocrine cells.[18] Some studies have shown that plasma CgA levels are increased in hypertension[19] and many other diseases such as myocardial infarction[20] and acute coronary syndrome.[21] A study has demonstrated that there is an important association between CgA and increased systolic and diastolic blood pressure, insulin resistance, high plasma triglycerides and high plasma total cholesterol.[22] These are defined as a part of the criteria for MetS. Genetic variation at CgA may influence the expression of genes in different populations. Thus, CgA may act on the inhibition of glucose-stimulated insulin release from pancreatic islet β- cells[23] and the inhibition of glucose uptake by adipocytes and hepatocytes.[24] A study recognized many single-nucleotide polymorphisms (SNPs) in the CgA locus.[25] Age and sex-related changes in CgA levels in patients with MetS are not exactly determined.[26,27] The CgA gene contains many polymorphisms in the promoter and coding regions. Functional polymorphisms C+87T (rs7610) and T-415C (rs9658635) may have an important role in hypertension and the pathogenesis of diabetes mellitus and regulating blood sugar, respectively.[28-31]

A study has shown that CgA is over-expressed in hypertensive humans and rodents.[32] It is also reported that CgA knockout (CgA-KO) mice are hypertensive.[33] Studies on humans and rodents reveal that aging correlates with insulin resistance and hypertension.[32] Thus, we decided to determine the possible differences of genetic polymorphisms T-415C (rs9658635) and C+87T (rs7610) and serum level of CgA that it may be affected by age and sex in the Fars ethnic group with and without MetS.

METHODOLOGY

Study subjects

The samples were collected from members of the native Iranian Fars ethnic group who were referred to the health center in Gorgan, Golestan province, Iran, and fulfilled the MetS criteria.[8] This study was done as an analytical case-control study. The sample size was conducted according to the 2008 Chen study[34] and frequency of polymorphism genotype C+^vT (p1= 0.59 and p2= 0.41) with a statistical confidence of 0.95 and power of 80 percent. The sample size was calculated at 117 in each group, and we also considered 5 percent missing. Finally, the sample size was considered 123 in each group and 246 in total.

$$n = \frac{(z_{1-\alpha/2} + z_{1-\beta})^2 (p_1q_1 + p_2q_2)}{(p_1 - p_2)^2}$$

Fifty-nine males and sixty-four females with MetS and sixty males and sixty-three females without MetS from the Fars ethnic group were included in this study. The study was carried out in the Metabolic Disorders Research Centre, Gorgan Faculty of Medicine, Golestan University of Medical Sciences. The Golestan University of Medical Sciences ethics committee approved our study (Ethic number: IR.GOUMS.REC.1400.096). Written consent was provided for all participants. Participants were excluded if they had liver disease, renal failure, lung disease, cardiovascular disease, acute and chronic infection, and inflammatory disease.

Ethics and consent statement

The Ethics Committee of the Research Deputy of Golestan University of Medical Sciences has approved the study (Ethics number: IR.GOUMS.REC.1400.096).

Experimental protocol

Anthropometric and laboratory measurements

Blood samples were extracted from all participants after a 12-hour fast and separated into two different tubes. Ethylenediaminetetraacetic acid (EDTA) was utilized as the anticoagulant to determine DNA extraction. Another part of the blood sample was used to measure serum fasting blood sugar (FBS), high-density lipoprotein-cholesterol (HDL-C) and triglycerides (TG) with commercial kits and spectrophotometric method. Waist circumference (WC) was determined in centimeters using a tape measure. Body mass index (BMI) was calculated by the formula weight (kg)/height (m)^2. Systolic and diastolic blood pressure (SBP and DBP) were determined by a digital blood pressure instrument. The definition of the subjects with MetS was done by Adult Treatment and the National Cholesterol Education Program Panel III (NCEP, ATP III).[8] According to this definition, five criteria were used for the diagnosis of MetS. The presence of three or more criteria was used for the diagnosis of MetS. The criteria are:

1-WC >102 cm (male), >88 cm (female)
2-TG >150 mg/dl
3- HDL-C <40 mg/dl (male), <50 mg/dl (female)
4- SBP and DBP >130/>85 mm Hg
5- FBS >110mg/dl

Single nucleotide polymorphism (SNP) assays

Whole blood was used to extract genomic DNA according to the salting-out method.[35] Two microtubes were utilized to separate the extracted DNA sample and stored at -20oC. Genotyping of the C+87T and T-415C was carried out by the T-ARMS-PCR (Tetra-primer Amplification Refractory Mutation System-Polymerase Chain Reaction) and PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) methods. The specific primer for T-415C and C+87T (the CgA gene) and their PCR product sizes are indicated as follows:

1-For +87 C>T:
1a-Forward inner primer (G allele):
481-GCCTCCCTACCGGAAGCATCG-501 with PCR product size 193 bp
1b-Control primer forward (5' - 3'):
382-GCCCTGCAAAGGATGTTCCAGG-403 with PCR product size 291 bp
1c-Reverse inner primer (A allele):
521-TCCTGGCCAGATGGCCCGTAT-501 with PCR product size 140 bp

1d-Control primer Reverse (5' - 3'): 672-GACCAGGAGCTGGAGAGCCTG-651 with PCR product size 291 bp
2-For -415 T>C:
2a- Common forward primer (5'-3'): CCTAGATATTGGAGAGAGCCATGAGTG
2b- Reverse (5'-3'): CCATGTGTACTGAGGTCCCTGGCAG with PCR product size 135 bp.

The total amount of mixture was 20 µl for T-ARMS PCR and RFLP PCR. The mixture consisted of forward and reverse primers with an inner to outer ratio of 1 to 1 and 2 to 1, 10 µl of PCR-master mix2X (sinaClon, Iran), 3 µl of DNAse free water and 2 µl of DNA template. The reaction condition consisted of initial denaturation at 95°C for 2 min, denaturation at 92°C for 30 sec, annealing at 64.5°C for 30 sec, and extension at 72°C for 50 sec followed by 32 cycles repeat steps 2-4 and a final extension at 72°C for 5 min. The scoring was done by running the PCR products on a 2% agarose gel electrophoresis over 40 min at 90 volts.

Validation of the genotyping results was done by the published method of PCR– RFLP which utilizes a BCCI enzyme (Ipswich, Massachusetts, United States, Cat. No. R0704S). The used reaction mixture was the same volume for forward and reverse primers with a ratio of 1 to 1. There were only differences in DNAse free water volume (2.5 µl). Two cycles of denaturation at 95°C for 2 min and then 30 sec, annealing at 65.8°C for 30 sec and cycles of extension at 72°C for 20 sec then repeat steps 2-4 to 38 times and the final step of extension for 72°C for 5 min were the initial program temperature. In the final step, 0.32 µl of enzyme, 2 µl buffer10x, 10.7 µl DNAse free water and 7 µl PCR products were mixed and given the temperature program mentioned above. 2.5% agarose gel electrophoresis stained by safe stain (Sina Clon, Iran) was done to determine PCR products.

Serum level of chromogranin A analysis

The enzyme-linked immunosorbent assay (ELISA) technique was used to measure chromogranin A using the Human CgA kit (Zell Bio GmbH, Cat.NO: ZB-11730C-H9648 Lot. No: ZB-OEH563210812-91, Germany).

Statistical analysis

The data was analyzed with the SPSS Statistical software (Version 23.0, Chicago for Windows) and was indicated as mean ± standard deviation and percentages. The Shapiro and Wilk test was used to determine the normality of quantitative variables. The Spearman and Mann-Whitney U tests were carried out to determine the correlation and the means of the quantitative variables between groups that did not have normal distributions, respectively. The chi-square test was used to compare qualitative variables. P-values less than 0.05 were considered significant.

RESULTS

Table 1 shows the demographic and biochemical variables of the males and females with and without MetS. The mean ages were 55.18 ± 3.34 and 57.27 ± 8.25; and 53.68 ± 3.25 and 56.43 ± 4.75 years in males and females with and without MetS, respectively. WC, SBP, TG, FBS and CgA levels were higher and HDL-C levels were lower when compared to females and males with MetS with those without MetS (P <0.001).

Table 2 shows the comparison of biochemical variables among the males and females with and without MetS. Males with and without MetS exhibited significantly lower HDL-C levels than those of females (P <0.001). Males without MetS showed significantly lower HDL-C in males with and without MetS when compared to females (P <0.001).

Table 1. Demographic and biochemical variables of the males and females with and without metabolic syndrome

Variable	Groups	Males		Females	
	MetS	Mean ± SD	*p*	Mean ± SD	*p*
Age (n)	MetS+	55.18 ± 3.34 (59)	0.435	57.27 ± 8.25 (64)	0.544
	MetS-	53.68 ± 3.25 (60)		56.43 ± 4.75 (63)	
FBS	MetS+	145.15 ± 45.63	**<0.001**	141.47 ± 43.84	**<0.001**
	MetS-	89.61 ± 5.21		89.79 ± 5.92	
TG	MetS+	208.03 ± 137.76	**<0.001**	158.47 ± 87.15	**<0.001**
	MetS-	82.33 ± 20.61		78.93 ± 21.16	
WC	MetS+	114.22 ± 9.65	**<0.001**	110.84 ± 12.32	**<0.001**
	MetS-	90.15 ± 11.15		84.85 ± 8.33	
SBP	MetS+	134.68 ± 1.83	**<0.001**	134.93 ± 1.85	**<0.001**
	MetS-	108.33 ± 1.05		108.34 ± 1.05	
DBP	MetS+	80.54 ± 1.14	0.056	82.45 ± 0.98	0.137
	MetS-	77.63 ± 1.07		75.12 ± 1.43	
HDL-C	MetS+	35.42 ± 6.35	**<0.001**	43.54 ± 7.54	**<0.001**
	MetS-	43.89 ± 6.66		51.35 ± 8.34	
CgA	MetS+	771.28 ± 158.48	**<0.001**	841.01 ± 612.23	**<0.001**
	MetS-	387.95 ± 98.60		384.82 ± 119.53	

WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; CgA: chromogranin A; Metabolic syndrome: MetS; MetS+ and MetS-: with MetS and without MetS

Tables 3 and 4 show age-related serum CgA and MetS components in males and females with and without MetS according to age distribution. For ages 30-39, 40-49, 50-59 and 60-70 years, females and males with MetS showed significant differences in FBS, TG and CgA levels and WC when compared to those without MetS (P <0.001). Females and males with MetS indicated significant differences in SBP at ages 40-49, 50-59 and 60-70 years, while at ages 40-49 and 50-59 years, there was a significant difference in HDL-C levels in males (P <0.001).

Table 5 shows the Spearman correlation between MetS components and serum CgA levels in the Fars ethnic groups with MetS according to sex. There was a statistically significant correlation between serum levels of FBS, TG, SBP and WC (in both sexes); and CgA in subjects with MetS (P <0.05). There was no significant correlation between DBP and HDL-C level; and CgA level in males and females, while there was a significant correlation between HDL-C level and DBP; and CgA level in males and females; respectively (P <0.001).

Table 6 shows CgA genotype frequency (T-415C and C+87T polymorphisms) in males and females with and without MetS. The frequencies of the CC, CT, and TT genotypes of C+87T were 3.4%, 32.2%, 64.6% and 0%, 23.3%, 76.6% in males and 3.1%, 31.2%, 65.6% and 0%, 27%, 73% in females with and without MetS, respectively (P >0.05). The

Table 2. Comparison of different variables among males and females with and without metabolic syndrome

Variable	Group	MetS-		MetS+	
		Mean ± SD	*p*	Mean ± SD	*p*
FBS	M	89.62 ± 5.21	0.834	145.15 ± 45.63	0.998
	F	89.79 ± 5.92		141.47 ± 43.84	
TG	M	82.23 ± 20.61	0.310	208.03 ± 137.76	0.095
	F	78.93 ± 21.6		158.47 ± 87.15	
WC	M	90.15 ± 11.15	0.055	114.22 ± 9.65	0.220
	F	84.85 ± 8.33		110.84 ± 12.32	
SBP	M	108.33 ± 1.05	0.202	134.68 ± 1.83	0.237
	F	108.34 ± 1.05		134.93 ± 1.85	
DBP	M	77.63 ± 1.07	0.823	80.54 ± 1.14	0.473
	F	75.12 ± 1.43		82.45 ± 0.98	
HDL-C	M	43.89 ± 6.66	**<0.001**	35.42 ± 6.36	**<0.001**
	F	51.35 ± 8.34		43.45 ± 7.55	
CgA	M	387.95 ± 98.60	0.958	771.28 ± 158.48	0.939
	F	384.82 ± 119.53		841.01 ± 612.23	

WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; CgA: chromogranin A; Metabolic syndrome: MetS; MetS+ and MetS-: with MetS and without MetS; M: Males and F: Females

Table 3. Age-related serum chromogranin A and metabolic syndrome components in males with and without metabolic syndrome

Ages	30-39 (years)		40-49 (years)		50-59 (years)		60-70 (years)	
Variables	MetS-	MetS+	MetS-	MetS+	MetS-	MetS+	MetS-	MetS+
HDL-C	44.50 ± 10.75	36.00 ± 6.05	43.28 ± 6.19	33.35 ± 5.43*	44.82 ± 7.04	35.48 ± 6.95*	41.35 ± 3.55	38.76 ± 5.47
FBS	59.25 ± 6.24	148.5 ± 43.86*	88.00 ± 6.54	165.94 ± 58.13**	90.31 ± 4.75	131.72 ± 26.09**	90.00 ± 4.03	142.84 ± 52.17**
TG	80.50 ± 14.38	227.25 ± 85.69*	81.06 ± 54.25	283.52 ± 193.03**	82.25 ± 20.08	191.12 ± 107.04**	85.55 ± 21.16	135.92 ± 53.54**
WC	91.25 ± 8.42	114.50 ± 3.87*	89.67 ± 13.49	111.35 ± 8.97*	90.78 ± 10.96	115.16 ± 11.45*	88.22 ± 9.93	116.08 ± 7.69*
DBP	78.70 ± 0.76	73.10 ± 1.19	79.30 ± 0.92	82.40 ± 1.44	76.30 ± 1.27	80.20 ± 1.02	79.10 ± 0.55	80.90 ± 0.89
SBP	122.25 ± 0.25	107.30 ± 1.19	108.70 ± 1.04	133.10 ± 2.17*	106.10 ± 1.00	134.70 ± 1.32*	109.40 ± 1.05	144.90 ± 1.327*
CgA	437.37 ± 50.84	838.48 ± 167.97*	352.73 ± 111.18	801.36 ± 208.72*	369.84 ± 103.48	757.37 ± 131.13*	393.02 ± 60.87	738.06 ± 132.23*

Mann-Whitney U tests were applied.
WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; CgA: chromogranin A; Metabolic syndrome: MetS; MetS+ and MetS-: with MetS and without MetS.
*P <0.05; **P <0.001

Table 4. Age-related serum chromogranin A and metabolic syndrome components in females with and without metabolic syndrome

Ages	30-39 (years)		40-49 (years)		50-59 (years)		60-70 (years)	
Variables	MetS-	MetS+	MetS-	MetS+	MetS-	MetS+	MetS-	MetS+
HDL-C	50.10 ± 0.11	44.50 ± 7.78	55.39 ± 9.84	42.67 ± 8.94	50.69 ± 7.78	42.78 ± 7.05	48.51 ± 6.77	46.54 ± 7.28
FBS	93.00 ± 2.84	143.00 ± 4.24*	88.75 ± 5.93	127.27 ± 16.16*	89.51 ± 6.19	143.06 ± 38.54*	91.27 ± 5.59	155.36 ± 77.59*
TG	48.00 ± 20.15	170.50 ± 57.27**	80.75 ± 28.18	168.13 ± 97.17**	75.58 ± 17.42	158.50 ± 60.86*	86.00 ± 18.24	143.00 ± 144.64*
WC	92.00 ± 1.08	114.50 ± 2.12*	83.19 ± 9.39	106.46 ± 13.04*	85.36 ± 7.01	112.08 ± 12.46*	85.13 ± 9.99	112.09 ± 11.62*
DBP	80.14 ± 0.98	79.01 ± 1.27	76.80 ± 1.28	81.20 ± 1.18	73.90 ± 1.68	82.50 ± 0.98	75.70 ± 1.27	84.60 ± 0.75
SBP	124.03 ± 1.35	125.50 ± 2.45	108.80 ± 1.15	129.10 ± 2.03*	107.70 ± 0.96	134.60 ± 1.69*	108.10 ± 1.13	143.90 ± 1.54*
CgA	518.19 ± 88.25	852.04 ± 107.71*	382.63 ± 138.41	795.85 ± 159.62*	375.52 ± 118.38	914.07 ± 803.59**	397.47 ± 105.25	661.45 ± 81.99*

Mann-Whitney U tests were applied.
WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; CgA: chromogranin A; Metabolic syndrome: MetS; MetS+ and MetS-: with MetS and without MetS.
*P <0.05; **P <0.001

frequencies of the TT, TC, and CC genotypes of T-415C were 52.5%, 42.4%, 5.1% and 76.7%, 21.7%, 1.7% in males (P = 0.021) and 65.6%, 34.4%, 0% and 52.5%, 25.4%, 0% in females with and without MetS, respectively (P >0.05).

Table 7 shows CgA genotype frequency (T-415C and C+87T polymorphisms) between males and females with and without MetS. CgA genotype frequency (T-415C and C+87T polymorphisms) showed no significant differences between males and females with and without MetS (P >0.05).

DISCUSSION

MetS is a serious public health problem in people of different countries.[36] It may be occurring because of the genetic polymorphism differences among different ethnic groups worldwide. It has been reported that CgA levels are mildly increased in different diseases such as hypertension, congestive heart failure, myocardial infarction, renal failure, and liver dysfunction.[37] The findings of this study indicated that there were significant differences in the serum CgA level and MetS components according to age and sex in the subjects

Table 5. The correlation of CgA with the MetS diagnostic criteria variables

Gender	Variable	HDL	FBS	TG	SBP	DBP	WC	CgA
Males	HDL-C	1.000	-0.503	0.0541	0.300	-0.072	0.432	-0.490**
	P-value	-	<0.001	<0.001	0.001	0.437	<0.001	<0.001
	FBS	-0.503	1.000	0.643	0.481	0.127	0.487	0.869**
	P-value	<0.001	-	<0.001	<0.001	0.170	<0.001	<0.001
	TG	-0.541	0.684	1.000	0.431	0.248	0.322	0.681**
	P-value	<0.001	<0.001	-	<0.001	0.006	<0.001	<0.001
	SBP	0.300	0.481	0.431	1.000	0.396	0.526	0.557**
	P-value	0.001	<0.001	<0.001	-	<0.001	<0.001	<0.001
	DBP	-0.072	0.127	0.248	0.396	1.000	0.041	0.079
	P-value	0.437	0.170	0.006	<0.001	-	0.657	0.392
	WC	0.432	0.487	0.322	0.526	0.041	1.000	0.672**
	P-value	<0.001	<0.001	<0.001	<0.001	0.657	-	<0.001
Females	HDL-C	1.000	0.494	-0.532	-0.206	-0.011	0.447	-0.467
	P-value	-	<0.001	<0.001	0.020	0.904	<0.001	0.072
	FBS	-0.494	1.000	0.634	0.463	0.212	0.598	0.882**
	P-value	<0.001	-	<0.001	<0.001	0.017	<0.001	<0.001
	TG	-0.523	0.634	1.000	0.227	0.117	0.266	0.584**
	P-value	<0.001	<0.001	-	0.010	0.190	0.003	<0.001
	SBP	-0.206	0.463	0.227	1.000	0.446	0.590	0.495**
	P-value	0.020	<0.001	0.010	-	<0.001	<0.001	<0.001
	DBP	-0.011	0.212	0.117	0.446	1.000	0.204	0.215**
	P-value	0.904	0.017	0.190	<0.001	-	0.021	0.015
	WC	0.447	0.598	0.266	0.590	0.204	1.000	0.664**
	P-value	<0.001	<0.001	0.003	<0.001	0.021	-	<0.0012

WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; CgA: chromogranin A; Metabolic syndrome: MetS

Table 6. CgA genotypes frequency (T-415C and C+87T polymorphisms) in males and females with and without MetS

Sex	Groups	C+87T			p	T-415C			p
		CC	CT	TT		TT	TC	CC	
Males	MetS- n (%)	0 0%	14 23.3%	46 76.6%	0.18	46 76.7%	13 21.7%	1 1.7%	**0.021**
	MetS+ n (%)	2 3.4%	19 32.2%	38 64.6%		31 52.5%	25 42.4%	3 5.1%	
Females	MetS- n (%)	0 0%	17 27.0%	46 73.0%	0.365	31 52.5%	16 25.4%	0 0%	0.269
	MetS+ n (%)	2 3.1%	20 31.2%	42 65.6%		42 65.6%	22 34.4%	0 0%	

CgA: chromogranin A

Table 7. CgA genotypes frequency (T-415C and C+87T polymorphisms) between males and females with and without MetS

Groups	Sex	C+87T			p	T-415C			p
		CC	CT	TT		TT	TC	CC	
MetS- n (%)	Males (n = 60)	46 76.7%	14 23.3%	0 0%	0.641	46 76.7%	13 21.7%	1 1.7%	0.536
	Females (n = 63)	46 73.0%	17 27.0%	0 0%		47 74.6%	16 25.4%	0 0%	
MetS+ n (%)	Males (n = 59)	38 64.4%	19 32.2%	2 3.4%	0.989	31 52.5%	25 42.4%	3 5.1%	0.098
	Females (n = 64)	42 65.6%	20 31.2%	2 3.1%		42 65.6%	22 34.4%	0 0%	

CgA: chromogranin A

with MetS compared to those without MetS. CgA genotype frequency (T-415C polymorphism) indicated significant differences in males with MetS when compared with those without MetS. CgA genotype frequency (T-415C and C+87T polymorphisms) showed no significant differences between males and females with and without MetS. Some studies have revealed that CgA may affect blood pressure, obesity, fat levels and pancreatic beta cells.[37-40] The study of Kogawa et al., on type 2 patients with diabetes, showed that the level of CgA in the saliva and serum of these patients was significantly higher than in the control group.[29] The exact mechanisms of aging and sex and their effects on genetic polymorphisms and serum levels of CgA are not completely understood in subjects with MetS. A study on the role of CgA in aging-related MetS showed that blood pressure increases in humans and mice as they age and insulin sensitivity and glucose tolerance decrease, but there was an opposite effect with aging in CgA-KO mice. Then, age may improve insulin sensitivity which can decrease the level of blood glucose. Decreasing blood glucose levels may improve change from a high of a normal blood pressure.[41] The mechanisms of CgA's effects on insulin sensitivity throughout aging are still not exactly clear. Age is an important factor in hypertension, death and cardiovascular death.[37] CgA level may be affected by aging itself and/or other age-related different diseases.[42] Ahmed et al.,[43] reported that increasing age was associated with higher CgA levels, which does not follow our findings that the CgA was increased in all ages and sex with MetS. Manaf et al.,[44] reported that serum CgA levels were significantly lower in obese children with MetS than controls with normal BMI which was not in agreement with our findings. A different expression of CgA in some diseases such as hypertension[45,46] makes it necessary to identify the genetic variants of CgA gene polymorphism that may control its gene expression.[47,48] Different ethnic groups may show different SNP frequency.[49-52] Variations in the regulatory regions may cause differences in gene expression among different ethnic groups.[53] Thus, we focused our study on the genetic variants of CgA gene polymorphism in the Fars ethnic groups according to age and sex.

We studied two polymorphisms (T-415C, C and C+87T). Our study showed that there is only one effect on CgA T-415C polymorphism genotype frequency in males with MetS in comparison to those without MetS. The CgA T-415C polymorphism genotype may be a risk factor for males in the development of MetS. CgA genotype frequency (T-415C and C+87T polymorphisms) showed no significant differences between males and females with and without MetS. A study of Chen et al.,[54] on hypertensive patients revealed that the CgA C+87T polymorphism expression is not only increased in hypertensive patients but also affected by the C+87T genotype. They followed the study of Zhang et al.,[30] that focused on patients with hypertension and showed that the CgA C+87T polymorphism had effects on blood pressure. Our findings showed no significant differences in CgA C+87T polymorphism genotype in both sexes with and without MetS. However, it looks like the elevation was restricted to males. A study revealed that in subjects with high blood pressure, males were significantly more effective than females.[54]

Mahapatra et al., could make normal the increased blood pressure of the CgA knockout mouse and exhibit the importance of CgA in homeostasis of blood pressure in living organisms. They showed that CgA can affect biochemical systems (at the tissue level) and cause changes in whole body systems (physiological systems). They also found that severe hypertension indicates insulin sensitivity and decreased triglyceride levels, and in that connection causes MetS.[33]

These findings were not in agreement with our findings, because our results did show a significant correlation between the systolic (in males), systolic and diastolic (in females) blood pressure, and CgA level in subjects with MetS. It may mean that blood pressure in females is more changeable to the variation of CgA level than in males, without consideration of genotype variations.

A study has revealed that human CgA is over-expressed in hypertension, and a genetic variant in the CgA C+87T genotype is strongly associated with human essential hypertension within the population.[54]

They have been shown that the sex-dependent effect of CgA genetics is different on blood pressure. They found that CgA secretion was increased in hypertension subjects. Their results revealed also that plasma CgA secretion is not only increased in hypertension but is also affected by the C+87T genotype.[54]

A study on the T–415C polymorphism of the CgA gene in diabetic patients[29] indicated that diabetic patients showed higher levels of CgA in saliva than in the control group.

The findings of Subramanian et al.,[55] about the CgA gene in the Indian population revealed that the T–415C polymorphism of the CgA gene significantly increases the CgA level. According to their findings, this may cause increased insulin resistance.[55]

This finding is in accordance with our results in males with MetS. The molecular mechanisms of how CgA gene polymorphisms may affect the serum level of CgA in type 2 diabetic patients are not entirely clear. The effect of CgA on glucose and lipid metabolism may influence insulin secretion. Thus, CgA may have a significant role in insulin resistance in both sexes.

Our study showed that CgA in males and females with MetS have a positive significant correlation with serum levels of FBS, TG, SBP and WC, while there was a negative and positive correlation between CgA and HDL-C (in males) and DBP (in females) with MetS, respectively.

Our findings also indicated that CgA gene polymorphism variations are more different in the T-415C genotype in

males than the C+87T genotype when compared to each sex with and without MetS. This may indicate that males with MetS may be more sensitive to the T-415C genotype variation than females.

The CgA knockout mouse showed a normal blood glucose level, despite the plasma insulin levels being 4.5-fold less than the level in wild-type mice. Thus, CgA may control insulin sensitivity and the CgA knockout mouse is hypersensitive to insulin.[55]

Plasma level of triglyceride is positively correlated with insulin sensitivity/resistance as one of the components of the MetS.[22,56]

The 1.3-fold decrease in plasma triglyceride level is firm with insulin sensitivity in the CgA knockout mouse.[56] The main source of triglycerides in our body is the liver, and triglyceride levels did not indicate any difference in CgA knockout and wild-type mice. This may imply that another tissue such as adipose tissue has an important role in the decrease in the level of triglyceride.[56]

CONCLUSION

The CgA appears to be strongly associated with MetS components in both sexes. Variation in CgA gene expression may affect the T–415C polymorphism in males. This may mean that the structure of CgA genetics differs in different ethnic groups. Differences in the serum level and expression of the CgA gene may show valuable study results that it may be expected a relationship between these variables and the MetS.

Data Availability
The data are not publicly available due to restricted information and the privacy of participants.

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

CRediT Author Statement
AM: Conceptualization, Investigation, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration; **NP:** Methodology, Formal analysis, Investigation; **AS:** Methodology, Formal analysis, Investigation, Data curation, Project administration; **MT:** Methodology, Formal analysis

Author Disclosure
The authors declared no conflict of interest.

Funding Source
This work has been supported by the Research Deputy of the Golestan University of Medical Sciences.

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ORIGINAL ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

Characteristics and Prevalence of Metabolic Syndrome Among Adult Filipinos with Hypothyroidism: A Cross-sectional Study*

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Abstract

Objectives. We determined the clinical characteristics and prevalence of metabolic syndrome among adult Filipinos with overt hypothyroidism.
Methodology. This is a cross-sectional study of 151 adults. Patients were recruited by sequential enrollment. Anthropometric and blood pressure measurements were performed followed by blood extraction for metabolic parameters and thyroid function tests. Clinical and laboratory characteristics were compared between patients with and without metabolic syndrome.

Results. The prevalence of metabolic syndrome is 40.4% (95%CI: 32.5%, 48.7%). Patients with metabolic syndrome have a waist circumference of 88.4 ± 7.7 cm in females and 93.3 ± 9.0 cm in males. The median fasting blood glucose was 111.4 (52.2) mg/dL, median systolic blood pressure of 120 (30) mm Hg and diastolic blood pressure of 80 (20) mmHg, median serum triglycerides of 174.3 (114.2) mg/dL, median HDL-C of 42.3 (19.2) mg/dL and a proportion of patients with diabetes (23.0%) and hypertension (44.3%), respectively. The presence of increased waist circumference is the most prevalent component seen among hypothyroid patients. There were no differences in terms of age, sex, etiology of hypothyroidism and anti-TPO levels in those with and without metabolic syndrome.

Conclusion. The prevalence of metabolic syndrome in adult Filipinos with hypothyroidism is high. Emphasis must be placed on early screening using waist circumference and metabolic parameters among hypothyroid patients who are at high risk of developing metabolic syndrome

Key words: dyslipidemia, hypothyroidism, metabolic syndrome, prevalence

INTRODUCTION

The presence of thyroid disease is associated with metabolic abnormalities due to the effects of thyroid hormones on nearly all major metabolic pathways.[1] Thyroid hormones are responsible for the regulation of basal energy expenditure through their effects on catabolism.[2] One of the most common metabolic abnormalities in overt hypothyroidism is dyslipidemia characterized by the increase in total and low-density lipoprotein cholesterol (LDL-C) levels and less often high-density lipoprotein cholesterol (HDL-C), serum triglycerides (TG), lipoprotein (a), apolipoprotein A1 and apolipoprotein B levels.[3-6] A similar pattern has also been seen among patients with subclinical hypothyroidism.[4-6] Of note, the total and LDL-C significantly improve after thyroxine replacement treatment in both forms of hypothyroidism. The dyslipidemia seen in hypothyroidism often coexists with other metabolic abnormalities including hypertension, insulin resistance and oxidative stress, all of them being risk factors for cardiovascular disease.[7-11]

Metabolic syndrome (MS) is a clustering of cardiometabolic risk factors that increase an individual's risk for atherosclerotic cardiovascular disease (ASCVD), stroke and diabetes mellitus (DM). The threshold criteria vary among definitions suggested by organizations such as the International Diabetes Federation (IDF), the National Cholesterol Education Programme Adult Treatment Panel

eISSN 2308-118x (Online)
Printed in the Philippines

Received: June 1, 2023. Accepted: July 30, 2023.
Published online first: February 8, 2024.
https://doi.org/10.15605/jafes.039.01.13

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* *International Travel Grant and Best Oral Poster Presentation, 40th Seoul International Congress in Endocrinology and Metabolism, October 28-30, 2022, Gwangju, South Korea.*

III (NCEP/ATP III) or the World Health Organization.[12] It is defined as the presence of at least 3 of the following features: (1) visceral obesity depending on race; (2) raised triglyceride level or on specific treatment; (3) reduced HDL cholesterol or on specific treatment; (4) raised blood pressure or on treatment of previously diagnosed hypertension; and (5) raised fasting plasma glucose, on drug treatment or previously diagnosed type 2 diabetes based on the IDF criteria.

Worldwide, the prevalence of MS in the general population ranges from 12.5% to 31.4% depending on the definition used.[13] In the Philippines, the prevalence of MS in the general population ranges from 11.9% to 51.0% depending on the criteria used.[12,14,15] Likewise, the prevalence of MS in hypothyroidism varies depending on the population: 36.19% in a recent retrospective cross-sectional local study in the Philippine General Hospital, Manila, Philippines,[16] 40.0% in Nigeria,[17] 53.24% in Southern India[18] and 51.8% in Venezuela[19]. In general, the prevalence of MS is higher among those with hypothyroidism compared to thyrotoxicosis.[17-20] These findings are supported by a study in Nepal looking at the prevalence of thyroid dysfunction among 169 adults diagnosed with MS by the NCEP/ATP III criteria where 26.6% have subclinical hypothyroidism, 3.5% have overt hypothyroidism, in contrast to only 1.7% having subclinical hyperthyroidism.[20] In the Philippines, the Philippine Thyroid Diseases Study (PhilTiDeS 1) showed that thyroid dysfunction was more common among women. The national prevalence of thyroid function abnormalities is 8.5%, true hypothyroidism and subclinical hypothyroidism have the prevalence rates of 0.4% and 2.2%, respectively.[21] However, there is still a paucity of local data and knowledge gaps regarding the prevalence of MS in patients with thyroid dysfunction[22], especially among Asians where we develop diabetes and cardiovascular diseases at much lower BMI.[23,24] Hence, in this study, we determined the clinical characteristics and prevalence of MS among adult Filipino patients with hypothyroidism seen in our outpatient thyroid clinic.

METHODOLOGY

Patients and procedures

A sequential enrollment of 151 patients diagnosed with newly diagnosed overt hypothyroidism was conducted in our outpatient thyroid clinic at the Philippine General Hospital. A minimum sample size of 86 patients was determined based on the formula

$$n = \frac{Z^2P(1-P)}{e^2}$$

where n is the sample size, Z is 1.96, the statistic corresponding to 95 % confidence (α of 0.05), P, 6.0% (past local study) is the prevalence and e, 0.05 is the assumed error estimate. We sequentially recruited patients starting from January 1 to December 31, 2021, during their outpatient consultation using the inclusion criteria: All adult patients who are 19 years old and above diagnosed with either (1) autoimmune hypothyroidism; (2) post-procedural hypothyroidism; (3) subclinical hypothyroidism; (4) central hypothyroidism; and (5) any of the aforementioned patients from 1 – 4 on glucose-lowering therapy or cholesterol-lowering drugs. All patients with the following: patients with active/untreated thyroid malignancies, amiodarone and drug-induced hyper- or hypothyroidism (except radioactive iodine) and all patients with any of the following comorbidities: chronic liver disease, kidney disease, congestive heart failure, pregnancy, patients on oral contraceptive pills, cancer chemotherapy and anti-retroviral therapy were excluded from the study. A summary of the patient recruitment flowchart is shown in Figure 1.

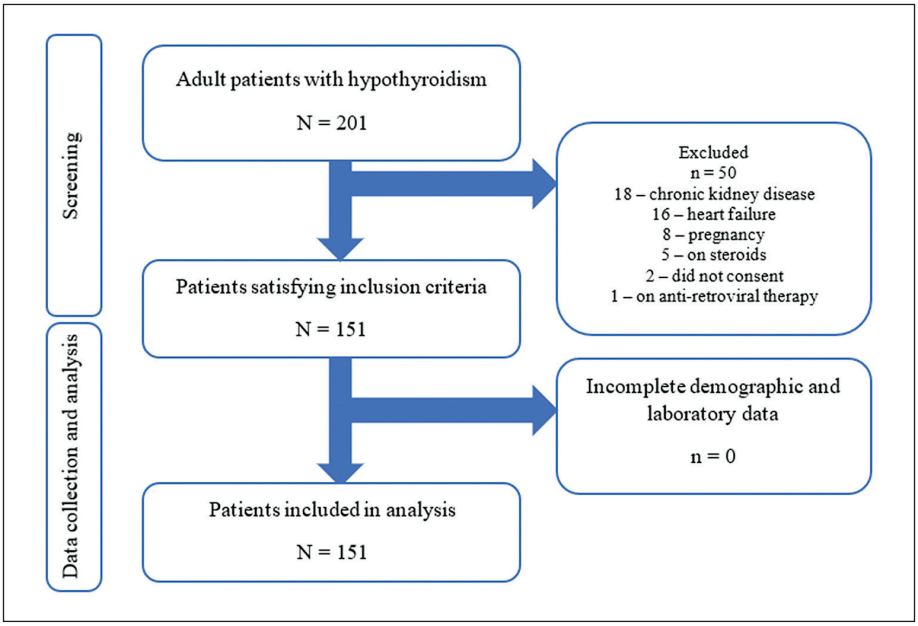

Figure 1. Flowchart of patient recruitment process.

Following informed consent, a trained research assistant obtained demographic data (age, sex, comorbidities – hypertension, diabetes mellitus, myocardial infarction, and stroke) using our approved data collection forms from patient interviews, patient's electronic medical records and physical chart entries. The same trained research assistant was assigned to measure anthropometric measurements [weight, height, body mass index (BMI), and waist circumference] and blood pressure. The anthropometric measurements were measured as follows: the patient's weight (rounded to the nearest tenth) and height (rounded to the nearest ones) were measured using the Detecto adult physician weighing and height scale, calibrated using known weights of 50 and 250 lbs, respectively; waist circumference (rounded to the nearest tenth) was measured using a tape measure in centimeters by passing the instrument through the umbilicus and using the bilateral iliac crests as landmarks; body mass index was calculated by dividing the patient's weight in kilograms by the patient's height in meters squared. Auscultatory blood pressure (rounded to the nearest ones) was measured in a seated patient using a calibrated desk-type Baxtel sphygmomanometer calibrated against an Omron digital sphygmomanometer after every 6 months of use. We used the first Korotkoff sound to mark the systolic blood pressure and the last Korotkoff sound as the diastolic blood pressure. All measurements were conducted 3 times and the average measurement was recorded in the data collection forms. This was then followed by venipuncture of 10 milliliters of blood. Blood collection for thyroid function tests and serum chemistry was performed prior to the initiation of thyroid hormone replacement. Five milliliters of blood contained in a red top tube was sent to the radioisotope laboratory for the measurement of the following: A. thyroid stimulating hormone (TSH), B. free thyroxine (FT4), and C. anti-thyroid peroxidase (anti-TPO) antibodies using the radioimmunoassay method (Beckman Coulter) while another 5 milliliters of blood contained in a red top specimen tube was sent to the medical research laboratory for the measurement of A. fasting blood glucose (FBS), B. total cholesterol (TC), C. high-density lipoprotein cholesterol (HDL-C), D. low-density lipoprotein cholesterol (LDL-C) and E. triglycerides (TG) using the colorimetric enzyme assay method. All blood samples were screened by our laboratory technician for adequacy prior to running. All laboratory personnel were blinded to the patient's clinical data.

Statistical analysis

All data collected were encoded, tabulated and summarized using Microsoft Office Excel 2016. Data analysis was performed using Stata version 17.0 with a p-value of less than 0.05 considered significant for all tests.

Clinical and laboratory characteristics of the hypothyroid patients were summarized by descriptive statistics. Numerical variables were described as mean and standard deviations (SD), if the data was normally distributed as assessed by the Shapiro-Wilk test for normality and as median and IQR, if otherwise. Categorical variables were described as counts and proportions. BMI was categorized using the WHO Asia Pacific classification while diagnosis of MS was determined using the IDF criteria.[12] The prevalence estimate of MS among adult patients with hypothyroidism was computed using a 95% confidence interval as the total number of patients satisfying the IDF criteria for diagnosis[12] divided by the total number of patients enrolled. The different clinical and laboratory characteristics were compared between the 2 groups: with MS and without MS. Significant differences in the mean waist circumference between the 2 groups were determined by two-way ANOVA to adjust for the blocking variable sex. Differences in means, medians or mean ranks between the 2 groups were determined by the Student t-test or Mann-Whitney U test, as appropriate. The heterogeneity of the proportions of the different categorical variables between the 2 groups was determined by the chi-square test. Thyroid function tests and thyroid autoantibody levels were also compared in the same manner. In addition, these thyroid tests were also compared according to the number of MS components that the patients have. The thyroid function tests and metabolic parameters were also compared among those with MS according to the etiology. One-way ANOVA was used for the normally distributed variables, while the Kruskal-Wallis test was used for otherwise. Post-hoc analysis by multiple comparisons using the Dunn method was used to identify the specific etiology that has significantly different TSH, FT4 and metabolic parameters.

Ethics

The study was approved by the Research Ethics Board of the University of the Philippines Manila (UPMREB CODE: 2020-605-01) prior to commencement and was conducted in accordance with the Declaration of Helsinki. All study participants provided written informed consent prior to any study-related procedures.

RESULTS

The baseline characteristics of our patients are summarized in Table 1. The male-to-female ratio was 1:2 (52:99 patients). There were no significant differences in terms of age. Primary hypothyroidism comprised 90.1% (n = 136) of the cohort; post-procedural causes comprised 76.2% (n = 115) and autoimmune causes at 13.9% (n = 21). Our results showed that the overall prevalence of MS among patients with hypothyroidism is 40.4% (95%CI: 32.5%, 48.7%). Among males, the prevalence is 40.4% (95%CI: 30.7%, 50.7%) while it is 40.4% (95%CI: 27.0%, 54.9%) among females.

Patients with MS had a waist circumference of 88.4 ± 7.7 in females and 93.3 ± 9.0 cm in males. The median fasting blood glucose was 111.4 (52.2) mg/dL, median systolic blood pressure of 120 (30) mm Hg and diastolic blood pressure of 80 (20) mmHg, median serum triglycerides of

174.3 (114.2) mg/dL, median HDL-C of 42.3 (19.2) mg/dL and a proportion of patients with diabetes (23.0%) and hypertension (44.3%), respectively (Table 1).

Among the different components of the MS among patients who fulfilled the diagnostic criteria, analysis showed that the component with the highest prevalence was the presence of an increased waist circumference at 86.9% (95%CI: 75.8%, 94.2%), followed by elevated triglyceride levels at 68.8% (95%CI: 55.7%, 80.1%), elevated blood pressure, hypertension or on specific treatment at 54.1% (95%CI: 40.8%, 66.9%), reduced HDL-C levels at 44.3% (95%CI: 31.6%, 57.6%) and elevated blood glucose, diabetes mellitus or on specific treatment at 44.3% (95%CI: 31.6%, 57.6%), respectively (Figure 2).

There were no significant differences between those with MS versus those without in terms of age, sex and anti-thyroid peroxidase antibody (anti-TPO) levels (Table 2). The number of components satisfied in the MS criteria in relation to the thyroid function tests (TSH, FT4) and anti-TPO levels are shown in Figure 3. Further analyses of

Table 1. Clinical characteristics of patients stratified based on age, sex, waist circumference, BMI, co-morbidities and etiology of hypothyroidism

Characteristics	Overall (n = 151)	(+) Metabolic syndrome (n = 61)	(-) Metabolic syndrome (n = 90)	*p*
Age (years), n (%)				0.015*
18 - 40	55 (36.4%)	14 (23.0%)	41 (45.6%)	
41 - 60	70 (46.4%)	33 (54.1%)	37 (41.1%)	
≥60	26 (17.2%)	14 (23.0%)	12 (13.3%)	
Sex, n (%)				0.998*
Female	99 (65.6%)	40 (65.6%)	59 (65.6%)	
Male	52 (34.4%)	21 (34.4%)	31 (34.4%)	
Waist circumference (cm), mean (sd)				<0.001**
Among females	83.2 (10.4)	88.4 (7.7)	79.8 (10.5)	
Among males	87.6 (9.6)	93.3 (9.0)	83.7 (7.9)	
BMI (kg/m²), n (%)				0.005*
<18.5	10 (6.8%)	1 (1.7%)	9 (10.2%)	
18.5 - 22.9	52 (35.4%)	13 (22.0%)	39 (44.3%)	
23.0 - 24.9	32 (21.8%)	16 (27.1%)	16 (18.2%)	
25.0 - 29.9	40 (27.2%)	21 (35.6%)	19 (21.6%)	
≥30.0	13 (8.8%)	8 (13.6%)	5 (5.7%)	
Comorbidities, n (%)				
Diabetes mellitus	19 (12.6%)	14 (23.0%)	5 (5.6%)	0.002*
Hypertension	35 (23.2%)	27 (44.3%)	8 (8.9%)	<0.001*
Myocardial infarction	-	-	-	-
Stroke	-	-	-	-
Etiology, n (%)				0.632*
Post-procedural	115 (76.2%)	44 (72.1%)	71 (78.9%)	
Autoimmune	21 (13.9%)	10 (16.4%)	11 (12.2%)	
Central	15 (9.9%)	7 (11.5%)	8 (8.9%)	
Fasting Blood Glucose (mg/dL), median (IQR)	90.7 (38.9)	111.4 (52.2)	91.6 (24.5)	-
Lipid profile				
Total cholesterol (mg/dL), median (IQR)	223.5 (92.6)	221.2 (123.4)	225.8 (75.8)	0.383
LDL_C (mg/dL), median (IQR)	139.2 (78.8)	126.5 (89.2)	142.9 (60.5)	0.138
Triglycerides (mg/dL), median (IQR)	140.7 (100.0)	174.3 (114.2)	118.6 (85.8)	-
HDL_C (mg/dl), median (IQR)				-
Among males	42.3 (18.8)	38.1 (19.5)	46.2 (19.6)	
Among females	53.5 (20.4)	43.6 (18.3)	57.6 (18.5)	
Non-HDL_C (mg/dL), median (IQR)	167.3 (80.2)	171.1 (111.7)	162.6 (68.1)	0.982
Blood pressure				
Systolic blood pressure (mmHg), median (IQR)	110 (30)	120 (30)	110 (20)	-
Diastolic blood pressure (mmHg), median (IQR)	80 (20)	80 (20)	70 (20)	-

* Student's t test
** Chi square test

Table 2. Baseline thyroid function tests and comparison of thyroid autoantibody levels between hypothyroid patients with and without metabolic syndrome

Thyroid tests	(+) Metabolic syndrome (n = 61)	(-) Metabolic syndrome (n = 90)	*p*
Thyroid function tests			
TSH (mIU/mL), median (IQR)	67.8 (90.5)	68.0 (72.0)	-
FT4 (ng/dL), median (IQR)	0.63 (0.93)	0.54 (0.64)	-
Thyroid autoantibodies			
Anti-TPO (IU/mL), median (IQR)	9.9 (18.8)	9.4 (9.3)	0.478*
Autoantibody positive, n (%)	10 (18.5%)	11 (13.4%)	0.420**

* Student's t test
** Chi square test

hypothyroid patients diagnosed with MS did not show any significant differences between thyroid function tests and metabolic parameters compared to the etiology of hypothyroidism (Table 3).

DISCUSSION

In this study, we determined the clinical characteristics and prevalence of MS among Filipino adults with hypothyroidism. We found that the prevalence of MS is high at 40.4% (95% CI: 32.5%, 48.7%) among patients with hypothyroidism. This value falls within the available international data on the prevalence of MS among patients with hypothyroidism (Table 4). While our results are consistent with our recent retrospective cross-sectional study conducted earlier in our institution,[16] our findings are in contrast to previous locally available data from Cebu City, where the prevalence was only 6.4% among 31 hypothyroid patients. We surmised that this relatively low prevalence of MS among those with hypothyroidism could be from several reasons. First, the exclusion of patients (n = 383) with incomplete data would have led to lesser inclusion of patients with hypothyroidism and subsequently, prevalence. Second, most patients who are stable on thyroid hormone replacement are less likely to seek consultation or follow-up compared to patients with very florid symptoms of hypothyroidism, which could also lead to fewer patients with hypothyroidism included at the onset. Third, as the status of hypothyroidism whether overt, subclinical or treated was not explicitly mentioned, the effect of thyroid hormone replacement on the improvement of metabolic parameters especially weight loss and lipid parameters could have also led to the decrease in prevalence.

Table 3. Clinical and laboratory characteristics of patients with metabolic syndrome and hypothyroidism

	Post-procedural (n = 44)	Autoimmune (n = 10)	Central (n = 7)	*p*
Thyroid function tests				
TSH (mIU/mL), median (IQR)	69.8 (74.2)	57.7 (93.8)	0.1 (0.9)	<0.001*
FT4 (ng/dL), median (IQR)	1.5 (0.2)	0.6 (0.7)	0.4 (0.8)	<0.001**
Metabolic parameters				
Waist circumference (cm), mean (sd)	90.1 (8.5)	90.2 (4.2)	90.1 (9.2)	0.999#
BMI (kg/m^2), mean (sd)	26.2 (3.1)	24.1 (3.3)	25.8 (3.9)	0.391#
Total cholesterol (mg/dL), median (IQR)	230.8 (134.8)	209.8 (95.5)	166.5 (92.8)	0.188#
LDL_C (mg/dL), median (IQR)	141.9 (96.7)	120.5 (87.3)	97.7 (76.7)	0.214#
Triglycerides (mg/dL), median (IQR)	192.0 (182.3)	155.4 (44.1)	185.1 (66.4)	0.318#
HDL_C (mg/dl), median (IQR)	42.0 (18.1)	44.0 (24.6)	38.5 (19.6)	0.908#
Non-HDL_C (mg/dL), median (IQR)	188.4 (121.5)	161.8 (88.7)	125.0 (68.8)	0.152#
Fasting blood glucose (mg/dL), median (IQR)	110.8 (61.5)	112.7 (30.8)	102.2 (40.9)	0.836#
Systolic blood pressure (mmHg), median (IQR)	120 (25)	130 (20)	130 (40)	0.487#
Diastolic blood pressure (mmHg), median (IQR)	80 (20)	90 (30)	80 (30)	0.265#

Student's t test
* Post-hoc analysis by Dunn test: central is significantly different from autoimmune (p <0.001) and primary non-autoimmune (p <0.001)
** Post-hoc analysis by Dunn test: central is significantly different from autoimmune (p <0.001) and primary non-autoimmune (p = 0.001)

Table 4. Comparison of studies on the prevalence of metabolic syndrome in the general adult population and adults with thyroid disorders

Author (Year)	Country	Population	Prevalence of metabolic syndrome	Reference
General Population				
Noubiap (2022)	Worldwide	N = 28,193,768 adults from 1,129 prevalence studies	12.5% by NCEP/ATP III criteria 28.2% by IDF criteria 29.1% by NCEP/ATP III-AHA/NHLBI criteria 31.4% by Joint Interim Statement (JIS) criteria	13
Punzalan (2004)	Philippines	N = 4541 (adults ≥20 years)	14.2% by NCEP/ATP III criteria 19.3% by International Atherosclerosis (IAS) criteria	12
Morales (2008)	Philippines	N = 4753 (adults ≥20 years)	11.9% by NCEP/ATP III criteria 14.5% by IDF criteria 18.6% by NCEP/ATP III-AHA/NHLBI criteria	14
Mata (2017)	Philippines	N = 1367	51.0% by NCEP/ATP III-AHA/NHLBI criteria 29.6% among normal BMI 18.5 – 22.9 kg/m^2 38.9% among overweight BMI 23.0 – 24.9 kg/m^2 56.9% among obese class I BMI 25.0 – 29.9 kg/m^2 62.4% among obese class II BMI ≥ 30 kg/m^2	15
Patients with thyroid dysfunction				
Chiu (2023)	Philippines	N = 105	36.19% among patients with hypothyroidism using Harmonizing definition (n = 38) 32.43% in men (n = 12) and 38.24% in women	16
Ogbera (2012)	Nigeria	N = 112	28.0% by NCEP/ATP III-AHA/NHLBI criteria 40.0% among patients with hypothyroidism by NCEP/ATP III-AHA/NHLBI criteria (n = 10) 24.0% among patients with hyperthyroidism by NCEP/ATP III-AHA/NHLBI criteria (n = 90) 42.0% among patients with non-toxic goiter by NCEP/ATP III-AHA/NHLBI criteria (n = 12)	17
Khare (2017)	Southern India	N = 154	53.24% among patients with hypothyroidism by NCEP/ATP III criteria (n = 82)	18
Bermudez (2018)	Venezuela	N = 391	56.8% among patients with subclinical hypothyroidism by IDF criteria (n = 41) No data available among euthyroid patients	19

Among the 5 components of MS in the patients who fulfilled the diagnosed criteria, the presence of an increased waist circumference had the highest prevalence at 86.9% (95%CI: 75.8%, 94.2%) while elevated fasting plasma glucose was the least common at 44.3% (95%CI: 31.6%, 57.6%). Our findings are consistent with worldwide data showing that the component with the highest global prevalence is ethnic-specific central obesity at 45.1% (95%CI: 42.1%, 48.2%), while elevated fasting glucose had the least global prevalence at 24.5% (95%CI: 22.5%, 26.6%).[13]

We also found that patients with central hypothyroidism have significantly higher FT4 levels compared to their primary hypothyroidism counterparts. This finding is consistent with the pathogenesis of central or secondary versus primary hypothyroidism where the primary pathology in the latter is the absent or decreased production of free thyroxine in the thyroid gland in contrast to an intact thyroid hormone synthesis in the former.[25-27] However, there were no significant differences between the etiology of hypothyroidism versus anthropometric measurements, clinical and laboratory parameters. This could be explained by the common final pathway of all forms of hypothyroidism which results in an overall decrease in the levels of FT4 and hence, resulting in similar lipid and metabolic abnormalities.[25-27] There were also no significant

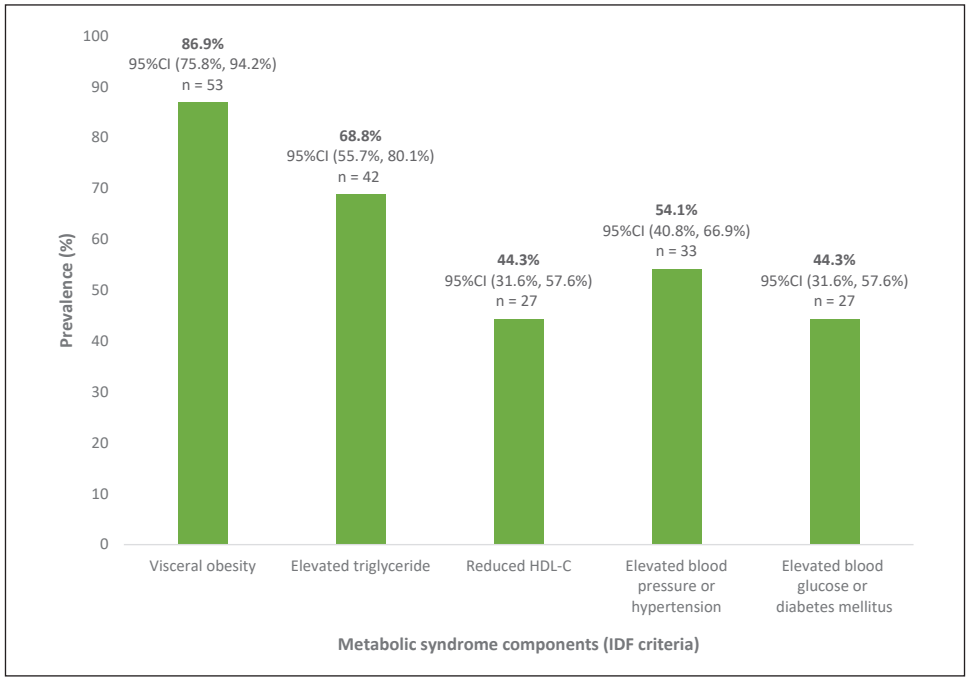

Figure 2. Prevalence of each component of metabolic syndrome among those who have satisfied the metabolic syndrome criteria.

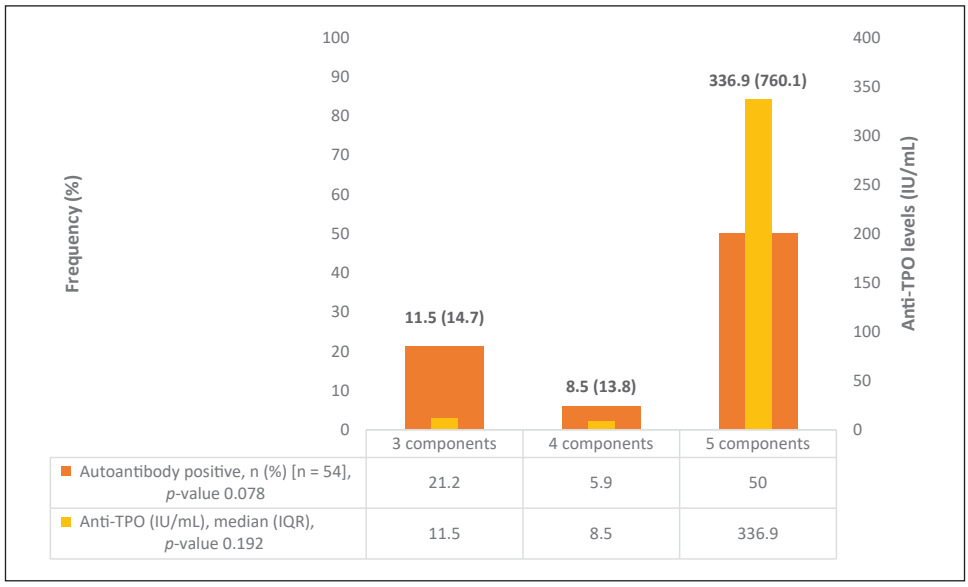

Figure 3. Thyroid autoantibody levels between number of metabolic syndrome components.

differences between those with MS versus those without in terms of age, sex, and anti-thyroid peroxidase antibody levels. This finding demonstrates that the driving force for the development of MS in a hypothyroid patient is mainly from the end-organ effects of the hypothyroid state itself. Hypothyroidism is characterized as a state of attenuated basal plasma insulin and insulin resistance which can increase cardiovascular risk, especially in the presence of increased waist circumference, increased body mass index, elevated SBP and/or DBP, elevated fasting plasma glucose, serum triglycerides and decreased HDL-C.[22]

The importance of our study was that we demonstrated that the prevalence of MS among adults with hypothyroidism is high. Our findings provide additional evidence and support early screening of our patients during clinic visits using waist circumference and metabolic parameters to identify patients with hypothyroidism who are at high risk of developing MS. It is interesting to note that screening for MS among patients with hypothyroidism has not yet been recommended as standard of care.[28]

Our study has several limitations. First, we were limited to a single center. Although we have surpassed our calculated sample size, we could have recruited more patients but we were limited by the prevailing COVID-19 pandemic situation where there was a major drop in the number of outpatient consultations. Second, patients with DM, on glucose-lowering therapy and those with dyslipidemia were included in the study. This might contribute to selection bias potentially leading to overestimation of prevalence. Ideally, patients without comorbidities present in the diagnostic criteria for metabolic syndrome should have been enrolled. Third, we did not include patients with subclinical hypothyroidism as this subset of patients is rarely encountered during consults and is mostly referred to us by other subspecialties when there are incidental thyroid function test result abnormalities. Lastly, we did not look into the effect of hormone replacement therapy and the quantification of the magnitude of changes in terms of anthropometric measurements, blood pressure, fasting blood glucose and serum lipids before and after hormone replacement therapy. The aforementioned limitations can limit the generalizability of our findings yet there can be new avenues for research looking into the effect of treatment on the metabolic profile of patients with hypothyroidism using a larger sample population with the inclusion of patients diagnosed with subclinical hypothyroidism.

CONCLUSION

In summary, our study showed that the prevalence of MS in adult Filipinos with hypothyroidism is high. The presence of increased waist circumference is the most prevalent component seen among hypothyroid patients. Hence, emphasis must be placed on early screening using waist circumference and metabolic parameters among hypothyroid patients who are at high risk of developing MS.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement

HHC: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **EQVIII:** Methodology, Validation, Formal analysis, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization; **RLJ:** Methodology, Investigation, Data Curation, Writing – review and editing; **AEA:** Methodology, Data Curation, Writing – original draft preparation, Writing – review and editing; **CJ:** Conceptualization, Methodology, Formal analysis, Resources, Data Curation, Writing – review and editing, Supervision, Funding Acquisition.

Author Disclosure

Cecilia Jimeno is the Vice Editor-in-Chief of the JAFES. The rest of the authors declared no conflict of interest.

Funding Source

The authors received research grants with a total amount of PhP 250,000: PhP 100,000 from the Philippine Lipid and Atherosclerosis Society and PhP 150,000 from the Philippine College of Endocrinology, Diabetes and Metabolism.

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ORIGINAL ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

Diagnostic Accuracy of American College of Radiology Thyroid Imaging Reporting Data System: A Single-center Cross-sectional Study

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Abstract

Objective. This study aims to evaluate the diagnostic accuracy of the American College of Radiology Thyroid Imaging Reporting Data System (ACR TI-RADS) in identifying nodules that need to undergo fine-needle aspiration biopsy (FNAB) and identify specific thyroid ultrasound characteristics of nodules associated with thyroid malignancy in Filipinos in a single tertiary center.

Methodology. One hundred seventy-six thyroid nodules from 130 patients who underwent FNAB from January 2018 to December 2018 were included. The sonographic features were described and scored using the ACR TI-RADS risk classification system, and the score was correlated to their final cytopathology results.

Results. The calculated malignancy rates for TI-RADS 2 to TI-RADS 5 were 0%, 3.13%, 7.14%, and 38.23%, respectively, which were within the TI-RADS risk stratification thresholds. The ACR TI-RADS had a sensitivity of 89.5% and specificity of 54%, LR + of 1.95 and LR - of 0.194, NPV of 97.7%, PPV of 19.1%, and accuracy of 58%.

Conclusion. The ACR TI-RADS may provide an effective malignancy risk stratification for thyroid nodules and may help guide the decision for FNAB among Filipino patients. The classification system may decrease the number of unnecessary FNABs for nodules with low-risk scores.

Key words: ACR TI-RADS, FNAB, thyroid nodules

INTRODUCTION

Thyroid nodules are among the most common endocrine pathologies in the general population. The incidence of thyroid nodules is increasing due to the wide use of thyroid ultrasound or other imaging tests that incidentally detect such nodules.[1] With high-resolution ultrasonography, thyroid nodule prevalence ranges from 19-68% in randomly selected individuals.[1,2] In the Philippines, the estimated prevalence of nodular goiter is 8.9% based on PhilTiDes 1 published in 2012.[3] Clinically, thyroid nodules are a significant cause of thyroid dysfunction and, rarely, compressive symptoms and malignancy in 7-15% of cases, depending on the risk factors.[4] Ultrasound is an essential, initial, and the most accurate imaging modality to detect and evaluate thyroid nodules. Specific ultrasound characteristics can identify which thyroid nodules are at higher risk of malignancy. Identifying the risk of malignancy is vital in managing thyroid nodules. It may guide clinicians if FNAB may be required.[1,2]

While fine needle aspiration biopsy is the preferred initial diagnostic method for evaluating thyroid nodules, only approximately 3-7% of thyroid FNAB are malignant.[5] This is why a better classification system is needed to stratify thyroid nodules based on ultrasound characteristics to lessen the number of nodules undergoing unnecessary FNABs. A systematic and reliable method to identify thyroid nodules with a higher risk of malignancy from those that may not need further invasive procedures may be of value.[1,2] There have been several guidelines for the standardization and stratification of nodules based on ultrasound findings.[6-8] The standardized risk stratification system was first introduced by Horvath et al., in 2009, called Thyroid Imaging, Reporting and Data System (TI-RADS), patterned from the BI-RADS system of breast imaging, and is now accepted by several societies.[9] This risk stratification of thyroid nodules based on several ultrasound features has been studied and established; however, it has yet to be universally adopted.[8] In 2017, the American College of Radiology released its paper on Thyroid Imaging

eISSN 2308-118x (Online)
Printed in the Philippines

Received: March 13, 2023. Accepted: May 23, 2023.
Published online first: January 8, 2024.
https://doi.org/10.15605/jafes.039.01.08

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Reporting and Data System (TI-RADS) that stratifies nodules according to a standardized set of terms (lexicon) for reporting sonographic features. These categories are assigned points in 5 ultrasound categories to determine their TI-RADS level.[9-12] The score aids in the decision to perform either biopsy or follow-up, recommending higher size thresholds for biopsy of less suspicious nodules and no biopsy on nodules of any size with benign features.[11] The need for a method to identify nodules that need FNAB is warranted since studies have shown that thyroid cancer has increased in incidence. However, this increase is partly due to screening thyroid sonography in asymptomatic patients causing over-diagnosis of thyroid cancer while mortality remains at a low rate.[11,13] In the study of Hoang et al.,[10] ACR TI-RADS guidelines significantly improved the accuracy of recommendations for nodule management, decreasing the number of thyroid nodules recommended for biopsy. Since invasive procedures for thyroid nodules cause a burden on healthcare costs and added anxiety to patients, risk stratification can aid in reducing unnecessary thyroid FNABs.

While FNAB is an accurate and practical method to evaluate thyroid nodules, a stratification system will guide clinicians to discuss and recommend evidence-based management and help patients understand and choose their options, providing better patient-centered management. Limited published studies were done in the Philippines that assess the validity and applicability of TI-RADS. More studies are warranted to test this stratification system to see its strength in identifying a nodule's malignancy risk. This study aims to evaluate the ACR TI-RADS risk stratification system in identifying nodules where FNAB can be safely deferred and its strength in identifying suspicious nodules in a single-center setting.

OBJECTIVES

General objective

To assess the diagnostic accuracy of the ACR TI-RADS in identifying nodules that need to undergo FNAB by comparing it to cytopathologic results of patients who underwent FNAB or thyroidectomy at St. Luke's Medical Center Global City, respectively.

Specific objectives

1. To identify specific thyroid ultrasound characteristics of nodules associated with thyroid malignancy in Filipinos in a single tertiary center.
2. To determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of ACR TI-RADS in detecting malignancy.

METHODOLOGY

Study design

This study is a single-center cross-sectional study approved by our institutional review board, and informed consent was waived.

Study population

Filipino patients aged 18 years and above who underwent ultrasound-guided FNAB or thyroidectomy at St. Luke's Medical Center Global City from January 2018 to December 2018 were included. Patients whose ultrasounds were performed in other centers were not included due to a lack of consistency in reporting and the unavailability of reviewable images. In addition, those without final cytopathology results or nodules with results of non-diagnostic, indeterminate, suspicious for malignancy (Bethesda category I, III, IV, V) with no repeat fine-needle aspiration biopsy or surgery done to confirm if the nodule is benign or malignant were also excluded. The FNAB results did include the side where the biopsy was done but did not include the specific location of the nodule. However, the best target nodule was selected using the description of the previous ultrasound report. All sub-centimeter nodules were not included in the TI-RADS assessment.

Sample size

The sample size was computed based on the prevalence of malignant nodules and sensitivity of the TI-RADS 4 based on the study of Horvath et al., which showed a prevalence of 76.13%, sensitivity 99.6% (95% CI: 98.9-100.0) and specificity 74.35% (95% CI: 68.7-80.0).[9] Using G*Power Application, the computed minimum sample size required was 145 with a margin of error of 5% and actual power of 95% based on the level of significance of 5%.

Study procedure

The ultrasound scans of the thyroid nodules included in the study were retrieved using the Radiology Information System - Picture Archiving and Communication System (RIS - PACS). Images were reviewed independently by two experienced sonologists blinded from the previous ultrasound report and cytopathology or histopathology result of the thyroid nodules. They stratified the thyroid nodule based on the set criteria of the ACR TI-RADS. Nodules were assigned points for each feature which were then summed up to determine the final TI-RADS score. The total number of points identified the nodule's ACR TI-RADS level and were subsequently categorized. The classification categories were graded according to their characteristic features in ultrasonography based on a TI-RADS point allocation scheme.[11] The nodules were classified as benign if the cytopathology result had a Bethesda score of II and malignant if they had a Bethesda score of VI. Nodules with Bethesda scores of III, IV, and V that underwent repeat

biopsy or were surgically removed were also included in the study if their repeat cytopathology or histopathology result returned as Bethesda II or VI or if benign or malignant, respectively.

Statistical analysis

The data were analyzed using Stata version 12 for Windows. The reference standard for malignancy in this study is cytology and histopathology results. The Shapiro-Wilk Test was used to check if the data is normally distributed. The mean age of patients with malignant and benign nodules was computed and compared using an independent t-test. Categorical variables were evaluated by chi-square test, including the patient sex and ultrasound features. For statistical correlation between pathologic results with ACR TI-RADS level, TR2 or TR3 were considered benign, and nodules with TR4 or TR5 as malignant.[14] To assess the accuracy of ACR TI-RADS in predicting a malignant thyroid nodule, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios were computed. Bivariate and multiple logistic regression analyses were performed to identify the sonographic features that are predictive of malignancy. Sonographic features with p <0.25 in the bivariate analysis were included in the multivariate regression analysis and significant features were selected using the backward-forward method. Crude and adjusted ratio and their corresponding 95% confidence intervals were determined. A p <0.05 will mean a significant contribution in predicting malignancy. A negligible amount of missing data was excluded from the analysis.

RESULTS

Study population

The total number included in this study was 176 nodules from 130 patients who had undergone a biopsy with cytopathology or histopathology between January to December 2018 at St. Luke's Medical Center-Global City (Figure 1). In this study, all subjects underwent FNAB while only eight proceeded with total thyroidectomy. The histopathology of the eight subjects who underwent thyroidectomy confirmed the FNAB cytopathology results. The mean age was 50 ± 14 (range 20-83). The mean nodule size was 2.17 cm ± 1.22 (range 0.6-6.7 cm). There were 19 malignant nodules (11%), with a mean size of 2.15 (SD 1.20). Table 1 summarizes patient demographics and characteristic ultrasound findings. Only records with available digital thyroid ultrasound images and those with definitive cytopathology or histopathology reports were included in this study. The most frequent characteristics of nodules on ultrasound were wider than tall (n = 172), smooth or ill-defined margins(n = 158), solid composition (n = 135), and none or large comet tail artifacts (n = 120). The cytopathologic diagnosis was reported using the Bethesda System of Classification.[7]

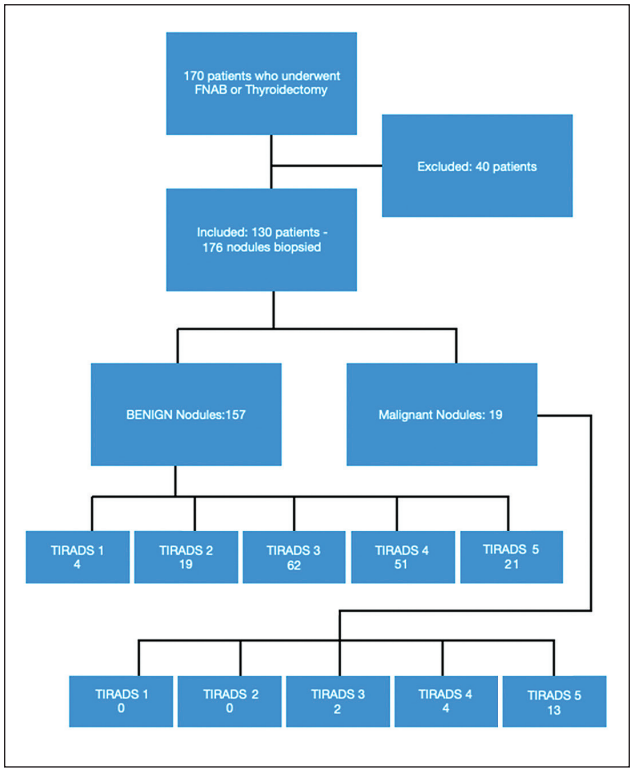

Figure 1. Flow of Participants.

Diagnosis of benign histopathology included benign follicular nodule goiter (i.e., adenomatous nodule, colloid nodule, etc.) (n = 139) and lymphocytic thyroiditis (n = 18). Malignant histopathology included Papillary thyroid carcinoma (n = 18) and Follicular thyroid carcinoma (n = 1). For malignant nodules, the mean age of the patients was comparable to those with benign nodules (mean, 48.79 ± 14.34 (range 20-83) vs. 49.41 ± 14 (range 20-81), respectively; P = 0.859). Gender was not statistically significant between malignant and benign lesions (P = 0.524). The mean size of both benign and malignant nodules was not statistically different as well (mean 2.12 ± 1.14 (range 0.5-6.7) vs. mean 2.15 ± 1.2 (range 0.8-4.20), respectively (P = 0.924). The chi-square test showed a statistically significant relationship between echogenicity, margin, and echogenic foci of the nodules (P = <0.0001). Binary logistic regression also showed that only echogenicity, margins, and echogenic foci are associated with malignancy, supporting the chi-square test result.

For echogenicity, there are fewer odds of malignancy specifically, 94% and 69% less [crude OR 0.06 (0.01, 0.26) and (crude OR 0.31 (0.10, 0.98), respectively], for patients with hyperechoic and hypoechoic echogenicity, respectively, compared with those with anechoic echogenicity.

A higher risk of malignancy is seen for margins as there are 13.36 times higher odds of malignancy (crude OR: 13.36, 95% CI: 3.97-44.98; p<0.0001) for patients with lobulated margins than those with lobulated margins with smooth/ill-defined margins. The odds ratio for malignancy in patients with punctate echogenic foci compared with those without or with large comet-tail artifacts is 13.14

Table 1. Summary of demographic features and ultrasound features

	Benign (N = 157)	Malignant (N = 19)	*p*
Age, mean (SD)	49.41 (14.00)	48.79 (14.34)	0.859
20-29	12 (7.64%)	-	
30-39	33 (21.02%)	6 (31.58%)	0.785
40-49	33 (21.02%)	4 (21.05%)	
50-59	44 (28.03%)	4 (21.05%)	
60-69	22 (14.01%)	3 (15.79%)	
70 and above	13 (8.28%)	2 (10.53%)	
Sex			0.524
Female	130 (83.87%)	17 (89.47%)	
Male	25 (16.13%)	2 (10.53%)	
Composition			0.884
Cystic or almost entirely cystic / Spongiform	9 (5.73%)	-	
Mixed solid and cystic	20 (12.74%)	2 (10.53%)	
Solid or almost completely solid	128 (81.53%)	17 (89.47%)	
Echogenicity			<0.0001*
Anechoic	4 (2.55%)	-	
Hyperechoic or isoechoic	90 (57.32%)	3 (15.79%)	
Hypoechoic	48 (30.57%)	8 (42.11%)	
Very hypoechoic	15 (9.55%)	8 (42.11%)	
Shape			0.369
Wider than tall	154 (98.09%)	18 (94.74%)	
Taller than wide	3 (1.91%)	1 (5.26%)	
Margin			<0.0001*
Smooth / Ill-defined	147 (93.63%)	11 (57.89%)	
Lobulated or irregular	7 (4.46%)	7 (36.84%)	
Extrathyroidal extension	3 (1.91%)	1 (5.26%)	
Echogenic foci			<0.0001*
None or large comet tail artifacts	115 (73.25%)	5 (26.32%)	
Macrocalcifications	14 (8.92%)	1 (5.26%)	
Peripheral calcifications	7 (4.46%)	1 (5.26%)	
Punctate echogenic foci	21 (13.38%)	12 (63.16%)	
Size			
Mean (SD)	2.12 (1.14)	2.15 (1.20)	0.924*
Median (range)	1.8 (0.5-6.7)	1.8 (0.8-4.2)	

* Significant at $p < .01$

Table 2. Association of clinical features with malignant thyroid nodules on FNA

	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Age	1.00 (0.96, 1.03)	0.859	—	—
		0.785		
Sex		0.524	—	—
Female (reference)	1			
Male	0.61 (0.13, 2.82)			
Composition		0.884	—	—
Cystic or almost entirely cystic /Spongiform (reference)	1			
Mixed solid and cystic	0.75 (0.16, 3.51)			
Solid or almost completely solid	—			
Echogenicity		<0.0001		0.073
Anechoic (reference)	1		1	
Hyperechoic or isoechoic	0.06* (0.01, 0.26)		—	
Hypoechoic	0.31* (0.10, 0.98)		—	
Very hypoechoic	—		3.33 (0.89, 12.44)	
Shape		0.369	—	—
Wider than tall (reference)	1			
Taller than wide	2.85 (0.28, 28.88)			
Margins		<0.0001		0.055
Smooth / Ill-defined (reference)	1		1	
Lobulated or irregular	13.36* (3.97, 44.98)		4.20 (0.97, 18.15)	
Extrathyroidal extension	4.45 (0.43, 46.46)		—	
Echogenic foci		<0.0001		0.000
None or large comet-tail artifacts (reference)	1		1	
Macrocalcifications	1.64 (0.18, 15.09)		—	
Peripheral calcifications	3.29 (0.34, 32.08)		—	
Punctate echogenic foci	13.14* (4.19, 41.19)		7.39* (2.41, 22.65)	
Size	1.02 (0.68, 1.54)	0.924	—	—

— Not applicable or not estimable.
* Significant at $p < 0.05$

Table 3. Diagnostic performance of ACR TI-RADS in predicting malignant thyroid nodules (n = 176)

Performance Indicator	Value	95% CI
Prevalence	11%	6.6%-16.3%
Sensitivity	89.5%	66.9%-98.7%
Specificity	54.1%	46%-62.1%
ROC area	0.718	0.637-0.799
LR +	1.95	1.55-2.45
LR -	0.194	0.052-0.727
PPV	19.1%	11.5%-28.8%
NPV	97.7%	91.9%-99.7%
Accuracy	58%	50.30%-65.34%

* Significant at $p < 0.01$

Table 4. Risk of malignancy by ACR TI-RADS category

ACR TI-RADS	Benign	Malignant	Calculated malignancy rate	*p*
TR1	4	0	0%	<0.001*
TR2	19	0	0%	
TR3	62	2	3.13%	
TR4	51	4	7.27%	
TR5	21	13	38.24%	

Table 5. Comparison of risk of malignancy predicted in ACR TI-RADS

ACR TI-RADS	Risk of malignancy [%]		
	ACR predicted (%)	Middleton et al.,[18]	Our study
TR1	<2	0.3	0.0
TR2		1.5	0.0
TR3	5	4.8	3.13
TR4	5.1-20	9.1	7.27
TR5	>20	35.0	38.24

(crude OR: 13.14, 95% CI: 4.19-41.19; $p = 0.0001$). On the other hand, when all factors were considered in a single model to account for possible confounding factors, echogenicity, margin and echogenic foci were included in the final model. Furthermore, only the echogenic foci were significantly associated with malignancy while controlling for echogenicity and margins. Specifically, there are 7.39 times higher odds of malignancy (adjusted OR (aOR) 7.39, 95% CI 2.41-22.65; p <0.001) for patients with punctate echogenic foci than those with no echogenic foci or with large comet-tail artifacts.

Comparing TI-RADS with histopathology, the diagnostic performance of ACR TI-RADS was as follows: sensitivity was 89.5%, specificity of 54%, LR (+) of 1.95 and LR (-) of 0.194, the NPV of 97.7%, the PPV of 19.1% and an accuracy of 58% (Table 3).

Table 4 shows that the malignancy rate for TI-RADS 2,3,4 and 5 was 0%, 3.13%, 7.41%, and 39.39%, respectively, which shows a statistically significant malignancy risk across TI-RADS 2 to 5 (P <0.0001).

DISCUSSION

Ultrasound features have been widely used in predicting the risk of malignancy of thyroid nodules. When taken in isolation, the sensitivity of ultrasound features is predictive of malignancy at 26.7 to 63%, which is not a reliable guide as to when to do FNAB.[11] The ATA guidelines recommend evaluating and managing patients with thyroid nodules based on ultrasound patterns and FNAB results. Individual sonographic characteristics were combined with categorizing ultrasound patterns of thyroid nodules into high suspicion, intermediate suspicion, low suspicion, very low suspicion, and benign pattern. Currently, the ATA recommends biopsy on nodules with ultrasonographic features as follows: (1) thyroid nodules measuring 1 cm and above with intermediate to high sonographic pattern; (2) thyroid nodules with low suspicious pattern measuring 1.5 cm and above; and (3) thyroid nodules with very low suspicious ultrasound pattern measuring 2 cm and above.[1]

In 2009, Horvath et al., introduced TI-RADS classification intending to improve the characterization of nodules based on ultrasound features and establish a scoring that would help determine which nodules need to undergo FNAB. Initially, they described ten ultrasound patterns of thyroid nodules with related risk of malignancy.[9] However, due to the complexity of the ultrasound patterns, it only applied to some thyroid nodules, and hence, cumbersome to use in clinical practice.

Over the years, several publications and different guidelines were proposed by different institutions to establish recommendations on ultrasound examination

of thyroid nodules. In 2014, Kwak et al., investigated a more practical classification of thyroid nodules. They used sonographic characteristics predictive of malignancy such as solid, hypoechogenicity, marked hyperechogenicity, microcalcification, micro-lobulation or irregularity of borders and taller-than-wide shape enabling them to classify nodules into five risk levels, TI-RADS 1 to 5.[15] In 2015, the American College of Radiology developed a standard lexicon practical for describing the sonographic characteristics of thyroid nodules that aim to risk stratify and triage nodules for consistent follow-up. They gradually refined and chose terms that demonstrated consistency regarding performance in describing thyroid nodules as to diagnosing thyroid cancer or classifying a nodule as benign. Allocated points for the different characteristics were based on the likelihood of being associated with malignancy.[16] In 2017, the published ACR TI-RADS enabled easier use among various readers of varying levels of expertise, primarily aiming to decrease the number of unnecessary thyroid nodule biopsies while identifying those that may need further investigation.[11]

A local study in 2017 assessed the accuracy of KWAK-TIRADS in stratifying the risk of malignancy in a single-center setting. The study showed that the solid nodule is the most frequently associated feature predictive of thyroid malignancy and that the higher the TI-RADS score, the higher the risk of malignancy.[17] In our study, sonographic features that showed a significant association with malignancy (Table 2) were echogenicity, margins and echogenic foci. Specifically, there were 94% and 69% fewer odds of having malignancy for patients with hyperechoic and hypoechoic echogenicity with crude OR of 0.06 (0.01, 0.26) and 0.31 (0.10, 0.98), respectively, compared to those with anechoic echogenicity. There are 13.36 times higher odds of malignancy for patients with lobulated margins than those with smooth/ill-defined margins. Lastly, there are 13.14 times more odds of malignancy for patients with punctate echogenic foci than those with no/with large comet-tail artifacts. When all factors were considered in a single model to account for possible confounding from each factor, echogenicity, margins, and echogenic foci remained in the final model.

The echogenic foci factor was significant in terms of association with malignancy while controlling for echogenicity and margins. Specifically, the odds of malignancy for patients with punctate echogenic foci is 7.39 times that of those with no echogenic foci or large comet-tail artifacts. There have been several studies that have reported that punctate echogenic foci have high specificity for malignant nodules.[18-20] This finding is similar to a local study that was published in 2015, showing that microcalcification was the only significant ultrasound finding that had a significant correlation with malignancy with an odds ratio of 11.3, while a nodule with more than two ultrasound features predictive of malignancy was more likely to be malignant on cytopathology (p <0.001).[21] Another local study showed that microcalcification and irregular margins were significant predictors of thyroid malignancy, similar to international data.[22] We used the ACR TI-RADS classification system in this study, where the predicted malignancy risk for each classification was guided by a multi-center study by Middleton et al. (Table 5). The predicted malignancy for each TI-RADS is as follows: TR1 and TR2 nodules were predicted to have a risk of malignancy lower than 2%, and FNA was not recommended for these nodules. TR3, TR4 and TR5 nodules were predicted to have a malignancy risk of less than 5%, 5.1–20%, and greater than 20%, respectively.[25] In our study, the calculated malignancy rates for TI-RADS 2 to TI-RADS 5 are 0%, 3.13%, 7.14%, and 38.23% which are all within the TI-RADS risk stratification thresholds. However, this was lower than expected by the ATA guideline's recommended malignancy risk for high and moderate suspicion patterns (70-90% and 10-20%, respectively).[1] The calculated malignancy rates of the nodules TR2, TR3, TR4 and TR5 were statistically significant between categories (P <0.0001). With its high sensitivity of 89.5% and a negative predictive value of 97.7%, we can assume that ACR TI-RADS is a reliable way to screen patients with thyroid nodules to recommend whether a biopsy is needed, or close follow-ups may be done, decreasing the number of unnecessary thyroid nodule biopsies. Following the ACR TI-RADS scoring system in this study, FNAB can be avoided in 83 out of 176 nodules or 47.16% of all biopsied nodules.

On the other hand, five malignant nodules (2.84%) of all the nodules biopsied could have been missed. Five nodules not recommended for FNA in the TR5 category were malignant, while three out of the 20 TR4 nodules not recommended for biopsy were malignant. Of the three TR4 malignant nodules, no follow-up was recommended for one of the nodules, while the remaining two were recommended to have a follow-up. These nodules were not recommended for biopsy since the nodule did not reach the size threshold for biopsy. In ACR TI-RADS, the size threshold to recommend FNAB is for nodules with at least the following sizes: 2.5 cm, 1.5 cm, and 1.0 cm for TI-RADS 3, 4 and 5, respectively.[23] The study of Ito et al., showed that there was no difference in the outcomes between patients with biopsy-proven thyroid carcinoma with nodules of <1 cm who underwent surgery and those who did not undergo surgical intervention, proving that this low-risk papillary carcinoma has an indolent behavior and observation may be prudent for these thyroid nodules.[24] This study does not answer whether the same applies to Filipinos who reported having more aggressive thyroid cancer behavior.

A limitation of this study is that final diagnoses were primarily based on cytopathology results and not surgical histology. However, it has been noted that the probability of a false diagnosis is low at <3% and <1% for TI-RADS 2 and TI-RADS 5, respectively.[17] There may be selection bias as this is an observational retrospective study, rather than a cross-sectional criterion-referenced study where the data is collected in real-time or prospectively from each patient. Finally, only 8 subjects underwent thyroidectomy, so the

gold standard of findings taken from surgical pathology reports was not achieved in the greater majority of patients and the ultrasound results were only compared with the FNAB cytopathology reports. In addition, we included nodules biopsied based independently on clinicians' and referring physicians' judgment. During this study, we found that the ACR-TIRADS was not commonly used. Analysis of inter-observer variability was not conducted such that when a discrepancy between the readings was noted, the consultant's reading was followed.

CONCLUSIONS AND RECOMMENDATIONS

We found that the ACR TI-RADS may be a reliable tool in stratifying thyroid nodules, with its sensitivity of 89.5% and a negative predictive value of 97.7%. ACR TI-RADS can aid in deciding whether an FNAB is warranted or whether close follow-up may be recommended. This could potentially decrease the number of unnecessary thyroid biopsies for nodules with low-risk scores. Specifically, this study showed that hypoechogenicity, irregular margin, and, most significantly, punctate echogenic foci predict the malignant potential of a thyroid nodule. Since several local studies have already shown how strongly echogenic foci are correlated with malignancy, it could be recommended to set a higher score for this feature and probably create a specific scoring system for Filipinos with thyroid nodules. With the increasing familiarity of clinicians with the different classification systems used for thyroid nodules, the need for a single standardized approach to stratifying thyroid nodules is deemed necessary. Using a standardized stratification system will help radiologists and clinicians recommend evidence-based and patient-centered diagnostic and treatment options for our patients. For future studies, we also recommend specifically identifying the location of the nodule (upper, mid, or lower) being biopsied.

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

CRediT Author Statement
PAA: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **ET:** Validation, Investigation, Resources; **ALE:** Validation, Investigation, Resources; **MPDM:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration.

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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REVIEW ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

Interrelationship of Sarcopenia and Cardiovascular Diseases: A Review of Potential Mechanisms and Management

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Abstract

Sarcopenia refers to an age-related reduction of lean body mass. It showed a reciprocal relationship with cardiovascular diseases. Thus, it is imperative to explore pathophysiological mechanisms explaining the relationship between sarcopenia and cardiovascular diseases, along with the clinical assessment, and associated management. In this review, we discuss how processes such as inflammation, oxidative stress, endothelial dysfunction, neural and hormonal modifications, as well as other metabolic disturbances influence sarcopenia as well as its association with cardiovascular diseases. Moreover, this review provides an overview of both non-pharmacological and pharmacological management for patients with sarcopenia and cardiovascular diseases, with a focus on the potential role of cardiovascular drugs to mitigate sarcopenia.

Key words: sarcopenia, cardiovascular diseases, aging, inflammation

INTRODUCTION

The term sarcopenia originates from the Greek word *"sarx,"* meaning muscle, and *"-penia,"* meaning deficiency. Collectively, sarcopenia means muscle deficiency or the age-related decline in lean body mass.[1] This disease is associated with other outcomes, e.g. increased risks for falls, functional decline, frailty, and mortality. The European Working Group on Sarcopenia in Older People (EWGSOP) clinically defines sarcopenia as the presence of low muscle strength and mass or muscle quality.[2] It is important to note that the definition of sarcopenia is not limited to muscle wasting, but also includes the functional impairment associated with it, thus shifting toward a function-centered model of approaching sarcopenia. This definition has also been acknowledged and espoused by the Asian Working Group for Sarcopenia (AWGS) with the inclusion of certain cut-off values for measuring muscle mass and strength in Asians.[3]

Sarcopenia is a prevalent disease; however, the exact numbers are difficult to determine due to varying diagnostic criteria. The prevalence of sarcopenia in people in their 50s, 60s-70s, and 80s are around 1–33%, 5-13%, and 50%,[4] respectively. This follows the trend that skeletal muscle mass is said to decline at 40 years old at approximately 8% per year and accelerates with age. Other studies reveal that the prevalence of sarcopenia in individuals aged 65 years and older ranges from 12.6% to 17.5% with an average of 15.2% in Europe[5] and 30.3% for males and 29.3% for females in Korea.[6] Aside from age, other factors that may contribute to the rate of sarcopenia are ethnicity, lifestyle, and physical activity.[7]

Sarcopenia is also associated with other disease entities such as cardiovascular disease (CVD), renal insufficiency, metabolic syndrome, and nonalcoholic fatty liver disease, among others.[7-9] In particular, sarcopenia was associated with a higher risk of carotid atherosclerosis, myocardial infarction, and atrial fibrillation.[8] Another study conducted on Korean adults older than 65 years old revealed that there was a higher likelihood of CVD in those with sarcopenia.[6] Conversely, some studies show that there was an increased chance of being diagnosed with sarcopenia in adults with CVD. In one study, results show that the prevalence of sarcopenia in patients with CVD was as high as 16.9%.[9]

eISSN 2308-118x (Online)
Printed in the Philippines

Received: March 28, 2023. Accepted: May 30, 2023.
Published online first: October 27, 2023.
https://doi.org/10.15605/jafes.039.01.03

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The objective of this paper is to further discuss the pathophysiologic mechanisms linking sarcopenia and cardiovascular diseases. Although sarcopenia is an established disease entity, management options for it are relatively sparse. Therefore, this paper also aims to provide an overview of the potential role of cardiovascular drugs in the management of sarcopenia.

PATHOPHYSIOLOGIC MECHANISMS UNDERLYING THE INTERRELATIONSHIP BETWEEN SARCOPENIA AND CARDIOVASCULAR DISEASES

Cardiovascular diseases associated with sarcopenia

Atherosclerosis is a persistent inflammatory process of thickening, hardening, and loss of elasticity of arteries in various organs such as the heart, brain, and even skeletal muscle due to the development of lipid-laden lesions.[10] Sarcopenia has been associated with atherosclerosis with inflammation and oxidative stress as possible underlying mechanisms linking the two.[11] Likewise, there is an association between frailty, arterial stiffness, vascular endothelial dysfunction, and hypertension in the elderly.[12-14]

The association between coronary artery disease and sarcopenia has been demonstrated by the increased prevalence of sarcopenia in patients who have coronary artery disease and the prognostic value of sarcopenia in the course of coronary artery diseases. In a study done in China, 78 (22.6%) out of the 345 enrolled participants who were hospitalized for coronary heart disease were found to have sarcopenia.[15] Similarly, results in another study showed that sarcopenia was linked to a greater prevalence of myocardial infarction in people who were overweight or obese compared to those who had normal weight (10.0% vs 4.3%, P = 0.020).[16] Even higher prevalence rates were observed in Brazil where 64.6% and 35.4% of the 99 elderly patients with myocardial infarction presented with sarcopenia and sarcopenic obesity, respectively.[17]

It has been proposed that the factors that lead to the development of coronary artery disease such as atherosclerosis, hypertension, diabetes mellitus, and obesity may have a more direct association with sarcopenia. Results of a few studies have shown that diabetes mellitus is significantly associated with sarcopenia.[18,19] It has been postulated that the muscle dysfunction observed in diabetic patients is due to hyperglycemia, insulin resistance, and inflammation.[20] In particular, chronic hyperglycemia leads to the accumulation of advanced glycation end products that build up in skeletal muscle and cartilage, making them less flexible.[21]

Sarcopenia has also been observed to influence the progression of coronary artery disease. In a prospective study by Kim et al.,[18] 1928 patients with coronary artery disease who underwent percutaneous coronary intervention were followed up to assess the clinical significance of sarcopenia in the course of CAD. Serum biomarkers, specifically the ratio of serum creatinine to serum cystatin C (Scr/Scys) and the ratio of estimated glomerular filtration rate by Scys to Scr (eGFRcys/eGFRcr), were used to estimate muscle mass. Results of the study show that low muscle mass, as indicated by the decreased values of the surrogate markers, is significantly associated with increased 3-year mortality risk.

Lastly, sarcopenia and heart failure have been shown to be interrelated, each one influencing the development and progression of the other. In the studies investigating the impact of muscle wasting in patients with chronic heart failure, muscle wasting was found to be higher among patients with chronic heart failure (19.5%)[22] compared to otherwise healthy older individuals.[5,6] Additionally, in the same study by Fulster et al.,[22] results indicate that muscle wasting is more prevalent in males and older patients.

Pathophysiological mechanisms for sarcopenia and cardiovascular diseases

Common pathophysiologic pathways link cardiovascular diseases with sarcopenia. The main disease processes involved in the development of sarcopenia and CVD are inflammation, oxidative stress, endothelial dysfunction, neural and hormonal modification, malnutrition, and physical inactivity. Figure 1 shows these overlapping pathophysiological mechanisms and the interaction between cardiovascular diseases and sarcopenia.

Inflammation

Various cytokines and inflammatory markers that induce atherosclerosis and heart failure are associated with sarcopenia. Some of those cytokines are interleukin (IL)-6, IL-1, tumor necrosis factor-α (TNF-α), galectin 3, TNF receptor 1, and TNF receptor 2.[23] Inflammatory cytokines may act on muscle receptors, increasing muscle breakdown or impairing their reproduction by upregulating the catabolic pathways while downregulating anabolic proteins such as growth hormone.[24]

IL-6 has both pro- and anti-inflammatory properties; however, its pro-inflammatory properties dominate and are associated with cardiovascular diseases, with higher levels leading to higher morbidity.[25] IL-6 is found in abundance in atherosclerotic plaques as it is synthesized by arterial wall cells, namely macrophages, vascular smooth muscle cells, and endothelial cells.[25] This inflammatory state may increase arterial wall stiffness by decreasing elastin which may then further release inflammatory mediators.[26] Additionally, IL-6 can be proatherogenic by stimulating the proliferation of vascular smooth muscle cells and platelets as well as endothelium activation. Vascular smooth muscle proliferation is due to the effects of monocyte chemoattractant protein-1 which recruits monocytes and smooth muscles to atheromas. Endothelial activation leads to increased expression of cell adhesion molecules such as ICAM-1, VCAM-1, and E-selectin. These adhesion

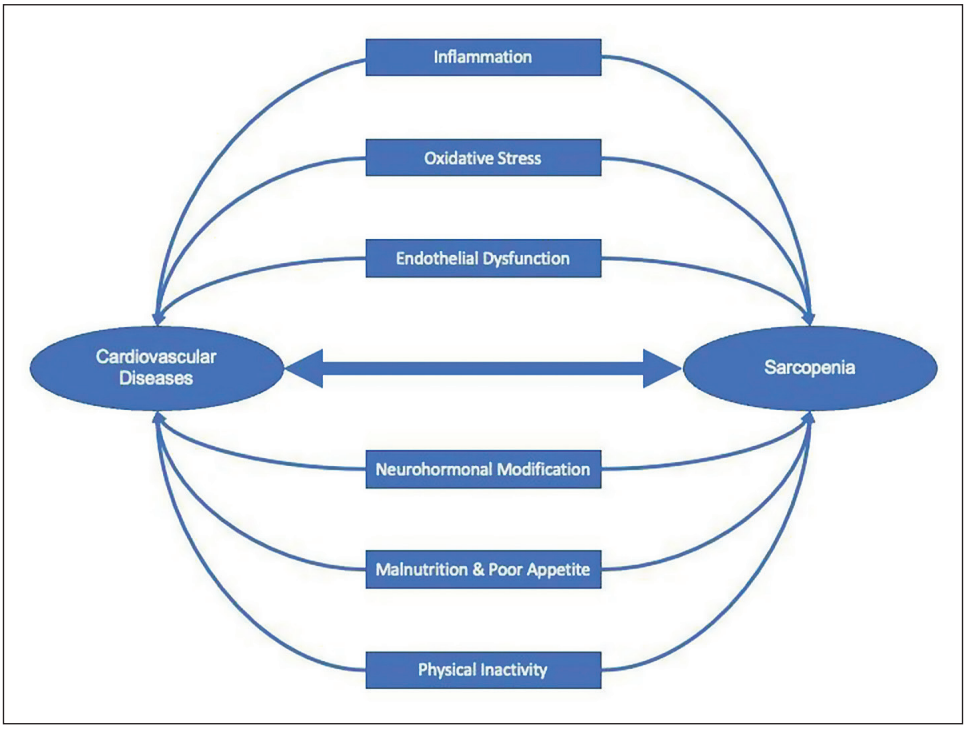

Figure 1. Pathophysiology of Sarcopenia and CVD and their interaction.

molecules then recruit monocytes that transmigrate into the subendothelium becoming macrophages and subsequently foam cells which produce atheromas.[25]

Furthermore, IL-6 levels can be increased by aging via several mechanisms. One mechanism is that aging increases bone marrow adiposity which stimulates the production of IL-6 and, subsequently, induces myeloid cell differentiation. The increased number of myeloid cells then recruits monocytes that are *TET*2-mutants that have a higher tendency for hematopoietic proliferation, thus leading to atheroma formation. Another mechanism is that aging enhances the production of IL-6 in vascular smooth muscle cells and decreases mitochondrial function. A decreased mitochondrial function affects mitophagy, or the removal of damaged mitochondria, which then increases IL-6.[27]

IL-6 is also a catabolic factor that promotes protein breakdown and muscle atrophy and inhibits muscle synthesis. Sarcopenia may be caused by IL-6 as this cytokine activates the ubiquitin-proteasome system which accelerates skeletal muscle breakdown.[28,29] In the study by Fulster et al.[22] in which serum cytokine levels of 200 patients with stable chronic heart failure were measured, those who presented with muscle wasting had significantly higher levels of IL-6 compared to those who did not have muscle wasting, furthermore implicating the role of inflammation in the pathogenesis of sarcopenia in the context of CVD. Indeed, it has been established that heart failure is a state of chronic low-grade inflammation.[23]

Another cytokine that links sarcopenia and CVD is TNF-α which is a key regulator in inflammation as it controls leukocyte activation, cytokine release, and production of free radicals.[30] Like IL-6, TNF activates endothelial cells to increase the expression of adhesion molecules which then recruit leukocytes. Moreover, TNF-α may promote endothelial cell injury and endothelial cell apoptosis, as well as decrease endothelial repair mechanisms, thus, leading to endothelial dysfunction. Endothelial cells may then recruit and activate leukocytes, induce apoptosis, and suppress endothelial cell progenitors, ultimately leading to atherosclerosis. Additionally, TNF-α may alter adipocyte metabolism which may result in atherosclerosis.[30]

One mechanism showing the association of TNF-α with sarcopenia is its potential to induce cell apoptosis via cysteine proteases called calpains, which are responsible for the proteolysis of proteins needed for cellular integrity, enzymes, and transcription factors.[31] Type II muscle fibers are said to be more susceptible to TNF-α-induced apoptosis. This apoptosis may be via the mitochondria-dependent internal pathway in which elevation in reactive oxygen species (ROS) and calcium (Ca^{2+}) levels in the cytosol affects mitochondrial homeostasis and increases cell permeability. Another pathway is the external pathway wherein TNF-α recruits adapter proteins on the cell surface which then activates a caspase cascade that leads to apoptosis, thus resulting in decreased muscle mass.[31]

Nitric oxide (NO) precursors are also known to reduce inflammation. Thus, a decrease in motor function due to chronic inflammation can be attributed to decreased NO signaling. Subsequently, sodium nitrite, a precursor of NO, was determined to attenuate the decline of motor function in mice by decreasing inflammation.[13]

Oxidative stress

Oxidative stress is known to create ROS that induces damage on various tissues.[7] Oxidized LDL-cholesterol (ox-LDL) is said to cause and induce atherosclerosis especially in the elderly due to the proinflammatory state seen in this age group. Oxidative stress may also lead to endothelial dysfunction which may increase the expression of endothelin-1 that promotes endothelium constriction, resulting in hypertension and atherosclerosis.[32] Furthermore, oxidative stress has not only been shown to be involved in cardiac remodeling in heart failure but also plays a role in the development of sarcopenia in these patients. The decreased cardiac output, endothelial dysfunction, and reductions in oxygen transport to the skeletal muscle that are implicated in the decline of muscle function in heart failure patients can all be linked to the generation of ROS.

Aging is associated with increased generation of ROS and decreased antioxidant production. Skeletal muscle is said to generate a large amount of ROS which induces post-transcriptional modifications. ROS also increases proteolysis and decreases protein synthesis leading to a decrease in muscle mass.[32] Additionally, increased production of ROS affects neuromuscular junctions (NMJ) by disrupting the homeostasis of Cu/Zn superoxide dismutase knockout mice motor neurons which also leads to disruption of skeletal muscle mitochondrial function. This increased ROS in skeletal muscle triggers a feedback mechanism that further affects the NMJ.[7] ROS also decreases acetylcholine release at the synaptic cleft, thus, leading to the failure of generation of an action potential. Furthermore, oxidative stress may influence NMJ physiology leading to a decrease in its innervation and muscle fibers, affecting the excitation-coupling mechanism, and altering the actin and myosin structures of muscle fibers – all of which may subsequently lead to sarcopenia.[32] Another proposed mechanism for the role of ROS in skeletal muscle dysfunction is ROS-induced insulin resistance which ultimately leads to decreased exercise tolerance.[33]

Aging cells have altered peroxisome proliferator-activated receptor-γ coactivator 1 α pathway which leads to an increased production of ROS which may, in turn, induce mitochondrial damage., and decrease the proliferation of skeletal muscle. The antioxidant sestrin has also been associated with sarcopenia and endothelial dysfunction with low levels correlating with low muscle mass.

Endothelial dysfunction and peripheral perfusion abnormalities

The pathogenesis of heart failure and hypertension may also involve endothelial dysfunction. Oxidative stress plays a role in endothelial dysfunction through the negative effects of reactive oxygen species on the availability of nitric oxide (NO), a powerful vasodilatory molecule, resulting in exaggerated vasoconstriction and decreased peripheral perfusion.[34] Since NO is responsible for blood redistribution during exercise through vasodilation of skeletal muscle arteries, its reduced availability in heart failure is associated with decreased exercise tolerance.[34] In a study evaluating sarcopenia and endothelial function in patients with chronic heart failure, results show that peak flow was significantly correlated with exercise capability in the forearm and leg.[35] Compared to patients without sarcopenia or controls, those with sarcopenia had reduced baseline forearm and leg blood flow. The observed exercise intolerance could be explained by the lack of oxygen delivery secondary to impaired blood flow. This is further illustrated by a decrease in the density of skeletal muscle capillaries and the ratio of slow, oxidative type I fibers to fast, glycolytic type II fibers.[29,36]

As aforementioned, NO is said to be an important cytokine in the dilation of blood vessels. Asymmetric dimethylarginine (ADMA) is a substance that inhibits endothelial NO synthase, an enzyme important in the generation of NO and vascular endothelial function, thus, leading to the inhibition of the NO signaling pathway and altering the homeostasis of vascular tone and arterial stiffness. A decrease in NO leads to an increase in vascular stiffness, which subsequently may lead to elevated blood pressure. Likewise, an increase in ADMA has been associated with an increase in cardiovascular mortality and is associated with frailty.[13]

Neurohormonal modification

Neurohormonal modification is also said to be involved in the pathogenesis of heart failure, myocardial infarction, and atherosclerosis. The enhanced activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS) in heart failure appears to be associated with increased catabolism of muscles.[36] The renin-angiotensin-aldosterone system may also be associated with sarcopenia and hypertension as sarcopenic patients were noted to have higher rates of urinary angiotensinogen excretion.[26] Angiotensin II is said to bind to angiotensin II type 1 receptor which then activates the PKC and/or Src pathway which subsequently leads to the activation of NADPH oxidase II which increases the production of reactive oxygen species. This may cause oxidative damage to muscle and increase protein catabolism and decrease protein synthesis. Elevated angiotensin II levels may also contribute to inflammation by increasing circulating glucocorticoids, IL-6, and serum amyloid A.[37] Furthermore, the role of RAAS in heart failure-related sarcopenia has been elucidated through the observed positive effects of angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin-II receptor blockers (ARB) on exercise and functional capacity.[36] However, there is sparse research on the benefits of mineralocorticoid antagonists such as spironolactone on the physical function of patients with heart failure.[36]

It was shown in rat models, that although the sympathetic nervous system has anabolic effects on skeletal muscle through B2-adrenoreceptors, chronic sympathetic stimulation could result in the downregulation of these receptors and, eventually, muscle wasting and atrophy.[38] To illustrate,

in a study assessing the role of autonomic modulation in 116 male patients with stable chronic heart failure, those with sarcopenia had higher muscle sympathetic nerve activity when compared to patients without sarcopenia.[39] The effects of sympathetic hyperactivity are further supported by the apparent efficacy of beta-blockers in slowing down catabolism while increasing the anabolism of skeletal muscles.[36] Furthermore, there are a few anabolic hormones that have been observed to be decreased in heart failure, namely growth hormone, testosterone, and ghrelin.[40] In another study, however, growth hormone levels are increased whereas insulin-like growth factor-1 (IGF-1) levels are notably lower in patients with sarcopenia and heart failure, implying that some form of resistance may be in play, resulting in less muscle formation.[41]

Patients with heart failure frequently have low testosterone levels which appear to be involved in the development of cardiac dysfunction.[40] Similarly, decreased testosterone levels have been shown to be linked to loss of muscle mass and function.[28] This is because testosterone may increase type 1 and 2 muscle fibers through increased insulin-like growth factor-1 (IGF-1).

Insulin resistance may also lead to arterial stiffness as insulin has anabolic effects on the skeletal muscle that increase endothelial-derived NO production leading to vasodilation.[26]

Additionally, levels of ghrelin, a hormone produced in the stomach that functions to increase appetite and food intake as well as promote growth hormone release, appear to be reduced in elderly patients with heart failure.[29] As nutrition and growth hormones have both been established to influence protein synthesis, it can be inferred that low ghrelin levels are associated with the development of sarcopenia in heart failure.

Myostatin, a protein belonging to the TGF-β family, is a strong inhibitor of the growth of skeletal muscles.[42] Enhanced expression of this protein from the heart is observed during pathological cardiac conditions such as myocardial infarction[43,44] and heart failure.[42] In a study where wild-type mice were compared with mice whose cardiomyocyte-specific myostatin (MSTN-CKO) was genetically deleted, skeletal muscle atrophy was associated with elevated serum myostatin levels in wild-type mice.[42] While myostatin serves as a compensatory mechanism by preventing further ventricular hypertrophy in heart failure, it also contributes to heart failure-induced muscle mass loss and, therefore, sarcopenia.

Malnutrition and poor appetite

Malnutrition is a frequent complication in patients with heart failure due to several factors such as poor appetite leading to decreased food intake, increased loss of nutrients from frequent diuresis, and elevated levels of inflammatory cytokines resulting in metabolic disturbances.[45] Commonly prescribed cardiovascular drugs, including digoxin, angiotensin-converting enzyme (ACE) inhibitors, β-blockers, and diuretics, have also been shown to cause nausea and dysgeusia, thereby negatively affecting appetite.[29] Heart failure could also be complicated by gastroenteropathy from intestinal edema which further promotes anorexia and malabsorption. Ultimately, these factors result in an imbalance between energy requirement and expenditure, favoring a catabolic state and loss of muscle mass.

Physical inactivity

In the elderly, decreased physical activity and exercise tolerance often lead to loss of muscle mass and, subsequently, increases the risk for obesity.[46,47] As obesity is linked to the increased production of pro-inflammatory markers such as adipokines and cytokines, infiltration of fat into muscle, and insulin resistance; the presence of obesity further exacerbates the development of sarcopenia, decreases physical performance, and increases the risk of mortality.[10,48,49] This vicious cycle between loss of muscle mass and gain of fat mass ultimately results in sarcopenia and sarcopenic obesity.

In heart failure, age-related decline in skeletal muscle mass and decreased cardiorespiratory fitness contribute to physical inactivity and exercise intolerance, further aggravating sarcopenia in these individuals. The significance of physical inactivity in the development and progression of sarcopenia is demonstrated by the efficacy of aerobic and resistance training in attenuating lean body mass loss and improving muscle function and strength.[29,50]

CLINICAL ASSESSMENT

Various diagnostic methods have been used to objectively measure skeletal muscle mass. Computed tomography (CT) and magnetic resonance imaging (MRI) estimate skeletal muscle area, dual-energy X-ray absorptiometry, and bioelectrical impedance analysis (BIA) determine body composition including lean body mass, and handgrip dynamometer and isokinetic dynamometer measure muscle strength. Physical performance can be evaluated by measuring the distance achieved in a 6-minute corridor walk and obtaining the time spent to finish a 4-meter or 6-meter distance walk. Supplementary measurements of inflammatory cytokines, hormone levels, and surrogate biomarkers (e.g., serum creatinine and serum cystatin) are also recommended to assess the other factors that could impact the development of sarcopenia.

Screening tests for sarcopenia have also been developed. The SARC-F is a tool that assesses five items namely strength, assistance in walking, rising from a chair, climbing stairs, and falls.[51] A recent version called SARC-CalF is a test that evaluates the aforementioned five items with an additional item on calf circumference. In particular, a calf circumference of ≤34 cm for males and ≤33 cm for females points toward sarcopenia.[52] A total score of ≥4 in SARC-F and ≥11 in SARC-CalF is a positive result for sarcopenia.

The European Working Group on Sarcopenia in Older People (EWGSOP) suggests that sarcopenia be diagnosed using the following criteria: 1) low muscle mass with a skeletal index of ≤8.90 kg/m^2 for males and ≤6.37 kg/m^2 for females, 2) low muscle strength with a handgrip strength of <30 kg for males and <20 kg for females, 3) low physical performance with a gait speed of ≤0.8 m/s. Sarcopenia may be called if there is low muscle mass with low muscle strength or physical performance.[53] The Asian Working Group for Sarcopenia (AWGS) in 2019 has also proposed updated cutoff values for sarcopenia in Asians: 1) muscle mass measurements of <7.0 kg/m^2 for males and <5.4 kg/m^2 for females using dual X-ray absorptiometry or <7.0 kg/m^2 for males and <5.7 kg/m^2 for females through bioimpedance analysis, 2) muscle strength estimates of <28 kg for males and <18 kg for females by handgrip strength, and 3) physical performance of <1.0 m/s gait speed in a 6-m walk.[54]

MANAGEMENT

Pharmacologic management

Cardiovascular drugs

Renin-Angiotensin-Aldosterone System Drugs

As aforementioned, the RAAS plays an essential role in maintaining cardiovascular homeostasis and is also interlinked with the development of decreased muscle mass. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARB) are frequently used medications for the treatment of cardiovascular diseases. Interestingly, a few studies have also demonstrated their muscle-protective properties through several mechanisms. Results of a study on mice by Marzetti et al., show that enalapril exerts its positive effects on muscles by reducing oxidative stress and inflammation.[55] ACE inhibitors are also shown to decrease loss in muscle strength in older adults without heart failure and increase muscle strength and exercise capacity, suggesting some potential in their use in sarcopenia.[56] In contrast, a recent systematic review and meta-analysis claim that the use of ACE inhibitors or angiotensin receptor blockers alone to enhance physical performance in the elderly is not supported by current research.[57] Additionally, a trial that aimed to determine the efficacy of leucine with or without perindopril compared to a placebo in improving physical function in adults of at least 70 years old with sarcopenia showed that neither enhanced physical performance.[58]

Similarly, another experiment illustrated that losartan protected against loss of muscle mass in rats by modulating the TGF-β signaling cascade and enhancing the insulin-like growth factor 1 (IGF-1)/Akt/mammalian target of rapamycin (mTOR) pathway.[59] The use of losartan in rat models also showed improved motility and reduced inflammation and oxidative stress.[56] Losartan intake and exercise in old mice also resulted to greater muscle mass and muscle fiber cross-sectional area which suggests that losartan may improve muscle mass and exercise capacity.[60] A longitudinal study conducted in aging populations showed that the use of ARBs was associated with greater frailty indices and increased calf circumference and composite muscle mass and strength.[61] A study also determined that elevated serum levels of losartan in pre-frail adults are associated with decreased frailty through a mechanism that is not dependent on the angiotensin II pathway which may suggest a role of losartan in maintaining physical function.[62] Furthermore, there has been a noted association in higher lean body mass in older females taking ACE inhibitors or ARBs which may suggest a protective function of these drugs for skeletal muscle mass.[63]

A few studies have shown the benefits of spironolactone, an aldosterone antagonist, in preventing skeletal myocyte apoptosis in rats and augmenting endothelial function and nitric oxide availability in patients with chronic heart failure.[64,65] However, recent studies have demonstrated that spironolactone, although effective as a treatment for cardiovascular diseases, did not significantly improve physical function and exercise capacity in patients with or without heart failure.[66,67]

Statins

Statins play a crucial role in preventing cardiovascular disease. It is an inhibitor of 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme involved in the production of low-density lipoprotein cholesterol, thus effectively lowering it. Statins are known to reduce cardiovascular events and improve mortality in patients with coronary artery disease or those at high risk for CVDs.[68] However, statin use is also associated with myopathies as statins increase the vulnerability of skeletal myocytes and induce the destruction of muscle proteins.[69] This may suggest that statin use may exacerbate muscle loss in patients. However, a cross-sectional study examining skeletal muscle volume in older adults taking a combination of ARB and statin is associated with a significantly higher skeletal muscle index.[70] Additionally, a study involving 136 patients with heart failure show that statin use was inversely associated with sarcopenia.[71] These studies suggest that statins may still have a potential role in maintaining muscle mass and further studies on their role in sarcopenia may be warranted.

Beta-blockers

Beta-blockers, another common cardiovascular medication, are postulated to prevent the progression of sarcopenia by inhibiting excessive sympathetic activation. In the COPERNICUS trial consisting of 2289 randomly assigned patients with heart failure, those in the carvedilol group were 33% less likely to lose weight (>6%) (95% confidence interval: 14-48%, P = 0.002) and 37% more likely to gain weight (>5%) (95% confidence interval: 12–66%, P = 0.002) compared to those in the placebo group.[72] However, another study suggests that this observed weight gain from the use of beta-blockers is mostly due to the increase in total body fat mass and content instead of an improvement in lean body mass.[73]

Therefore, more extensive research is required to fully understand the benefits of these drugs with a possible focus on dose adjustments of currently known regimens or the development of novel medications to target sarcopenia along with cardiovascular diseases.

Hormone replacement

Several hormones were investigated to assess their value in treating sarcopenia, but most remain to have inconclusive benefits. The role of growth hormone and IGF-1 in improving skeletal muscle mass and function has been established.[74,75] Thus, supplementation is being investigated as an alternative treatment option for sarcopenia. Testosterone administration to patients with chronic heart failure resulted in significant improvement in muscle strength, exercise capacity, and insulin sensitivity compared to those only given placebo.[76] The same findings were previously observed in another study by Pugh et al. in which males with chronic heart failure who were administered testosterone exhibited enhanced physical function in comparison to those receiving placebo.[77] Nevertheless, the effects of testosterone on the cardiovascular system have yet to be fully described. Selective androgen receptor modulators (SARMs) are another class of drugs under investigation mainly for their potential to increase lean body mass. A few studies have demonstrated their anabolic effects in healthy elderly males and postmenopausal females, in patients with cancer, and adult mice deficient in androgen activity.[78,79] However, studies with larger, more representative samples are needed to confirm their muscle-protective properties. The benefits of ghrelin were also examined in a rodent post-myocardial infarction chronic heart failure model by Barazzoni et al.[80] Administration of acylated ghrelin showed positive effects on skeletal muscle mitochondrial function, inflammation, and insulin activity. Vitamin D supplementation has been shown to help enhance muscle strength in healthy elderly individuals but its efficacy in improving muscle function and exercise capacity in patients with cardiovascular disease and sarcopenia has not been fully explored.[81]

Non-pharmacological management

Diet and nutritional intake

Several studies have demonstrated the importance of protein consumption for the maintenance and improvement of skeletal muscle mass and function. A few of these aimed to investigate the recommended amount of protein to be given per meal to optimize the dose-response relationship between dietary protein intake and myofibrillar protein synthesis.[82,83] According to Moore et al., compared to younger males, healthy elderly males require a relatively greater amount of protein per meal to maximize its potential anabolic effects.[84] Lancha et al.,[85] recommend 0.4 g protein/kg body weight per meal, equivalent to 1.2-1.6 g protein/kg body weight/day, for adequate protein synthesis in the elderly. This amount exceeds the daily recommended intake of 0.8 g protein/kg for younger individuals. Thus, the elderly must adhere to a dietary regimen that ensures increased healthy protein intake that will not compromise the status of existing comorbidities such as cardiovascular diseases. This increase in protein intake can be achieved by consuming whey and other dietary protein supplements that may provide bioavailable essential amino acids that may be used for protein synthesis. Furthermore, whey protein is noted to have high leucine content which has been shown to increase protein synthesis in animal models.[86]

Furthermore, supplementation of essential amino acids, specifically β-hydroxy-β-methylbutyrate (HMB), has been examined for its potential anabolic and anti-catabolic activity. Most studies about HMB claim that it is significantly more efficacious in reducing the risk of sarcopenia and in increasing muscle mass and strength when its supplementation is combined with exercise.[87-90] Still, its potential to mitigate muscle atrophy and improve muscle mass, albeit less prominent, is significant in the management of sarcopenia in bedridden and sedentary elderly individuals.[91]

Exercise

Aerobic exercise and resistance training have been demonstrated to improve muscle strength, exercise tolerance, and overall functional capacity in patients with cardiovascular diseases and have shown to be the only therapeutic strategy supported by adequate clinical data for treating muscle wasting in heart failure.[38] In a study by Pu et al.,[50] patients with chronic heart failure who underwent progressive resistance training exhibited significant improvements in skeletal muscle strength and endurance compared to those only performing low intensity stretching exercises. Several mechanisms behind these positive effects have been extensively researched which include enhancing IGF-I/Akt/mTOR signaling pathway, reducing levels of inflammatory cytokines specifically TNF-α, mitigating the catabolic effects of the ubiquitin-proteasome system, and decreasing the levels and activity of myostatin.[92-96]

CONCLUSION

Multiple studies support that a mutual relationship between sarcopenia and cardiovascular diseases exists. The pathophysiology behind this association is complex and

Table 1. Summary of key findings

- Sarcopenia is a pathologic loss of muscle mass and functional impairment associated with aging
- Sarcopenia is said to be associated with cardiovascular diseases (CVDs) such as atherosclerosis, hypertension, coronary artery disease, and heart failure
- Various mechanisms may link sarcopenia and CVDs but the two major underlying pathologic mechanisms are inflammation and oxidative stress. Other mechanisms that may contribute to sarcopenia may be decreased physical activity, neurohormonal modification, and malnutrition.
- There are several modalities that may mitigate the progression of sarcopenia and CVDs such as cardiovascular drugs, hormone replacement, modification of diet and nutritional intake, and exercise.

involves several systemic factors including inflammation, oxidative stress, endothelial dysfunction, neurohormonal signaling, and other metabolic disturbances. Sarcopenia in the elderly not only influences the development and progression of cardiovascular diseases but is also heavily affected by the presence and severity of these comorbidities. Thus, early screening and diagnosis of sarcopenia have relevant implications for the management of cardiovascular diseases and overall quality of life. There are several potential management options for sarcopenia. The pharmacologic approach includes utilizing angiotensin-converting enzyme inhibitors, angiotensin-II receptor blockers, mineralocorticoid antagonists, statins, beta-blockers, and hormone replacement. However, further investigation is required to fully comprehend their potential to treat sarcopenia, especially in the context of cardiovascular diseases. Another approach that has been proven to be effective is to introduce lifestyle modifications which include increasing dietary protein intake and engaging in regular exercise, particularly aerobic and resistance training. Overall, more comprehensive research has to be done to gain a clearer understanding of the mechanisms involved in sarcopenia and their implications on future therapeutic approaches.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement

FR: Conceptualization, Methodology, Validation, Resources, Writing - review and editing, Supervision, Project administration, Funding acquisition; **BTE:** Formal Analysis, Investigation, Data Curation, Writing - original draft preparation, Writing - review and editing, Visualization; **FMN:** Formal Analysis, Investigation, Data Curation, Writing - original draft preparation, Writing - review and editing, Visualization; **SC:** Writing - review and editing, Supervision, Project administration; **GPC:** Writing - review and editing, Visualization, Supervision; **EL:** Conceptualization, Methodology, Validation, Resources, Writing - review and editing, Supervision, Project administration**; KV:** Writing - review and editing, Visualization, Supervision; **MGY:** Writing - review and editing, Visualization, Supervision

Author Disclosure

The authors declared no conflict of interest.

Funding Source

None.

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REVIEW ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

A Systematic Review of the Accuracy of Insulin and C-peptide Secretion Ratios During the Oral Glucose Tolerance Test to Diagnose Insulinoma

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Abstract

Background. Insulinoma is one of the causes of recurrent hypoglycemia, one of the chief complaints for emergency department admission. The gold standard in diagnosing insulinoma is a 72-hour fasting test which is inconvenient and inefficient as it requires hospitalization. Research has found that measurement of insulin and C-peptide during OGTT may help diagnose insulinoma. We aimed to assess the diagnostic value of OGTT in diagnosing insulinoma.

Methodology. The literature search was conducted on 19 August 2022 using several databases (MEDLINE, Scopus, Embase, and ScienceDirect). All studies that measured OGTT as diagnostic tools in diagnosing insulinoma and 72-hour fasting test as reference standard were included. The quality assessment of the selected studies was based on the Centre of Evidence-Based Medicine University of Oxford and the Quality Assessment of Diagnostic Accuracy-2 tool (QUADAS-2). Analysis of the included studies was performed qualitatively. This study was registered on PROSPERO (CRD42022360205).

Results. A total of two case-control studies (106 patients) were included, which were at risk of bias and low concern of applicability. Both studies demonstrated that the combination of insulin and C-peptide levels measured during OGTT had high specificity, sensitivity, positive predictive value, and negative predictive value in diagnosing insulinoma compared to the reference standard. A logistic regression model of 8.305 – (0.441 × insulin 2-h/0-h) – (1.679 × C-peptide 1-h/0-h) >0.351 has the highest diagnostic value in one study (AUC 0.97, Sensitivity 86.5%, Specificity 95.2%, PPV 94.1, NPV 88.9).

Conclusion. The measurement of 0-h and 2-h insulin and C-peptide levels during 2-h OGTT was found in two small case-control studies with a total of 106 patients to have good sensitivity and specificity. However, due to these limitations, future research is still needed to validate the potential use of OGTT for the diagnosis of insulinoma.

Key words: hypoglycemia, insulinoma, 72-h fasting test, oral glucose tolerance test

INTRODUCTION

Hypoglycemia is one of the major reasons for Emergency Department admissions.[1] Hypoglycemia is characterized by: (1) low blood glucose level (<50 mg/dL), (2) signs or symptoms of hypoglycemia, and (3) alleviation of signs or symptoms following treatment, known as the Whipple's triad.[2] The most frequent etiology of the admission is the usage of hypoglycemic agents in diabetes mellitus patients. Endocrine disorders, malignancies, malnutrition, and renal insufficiency are among the other causes of hypoglycemia in non-diabetic patients.[1] In one study of 1196 episodes of hypoglycemia, most of the episodes (69.3%) happened in diabetic patients. While the other episodes (30.7%) happened in non-diabetic patients, 9.28% were due to malignancies.[1] The most common functioning pancreatic neuroendocrine tumor is an insulinoma.[3]

Insulinomas account for 1-2% of all pancreatic tumors. It occurs in 1 to 4 people per million people annually.[3,4] More frequently, insulinomas present as a single benign tumor. However, an insulinoma may be malignant in 5.8% of cases and 6% to 7.6% of cases linked with MEN1 syndrome.[4]

To this date, the gold standard in diagnosing insulinoma is the induction of a symptomatic hypoglycemia state

eISSN 2308-118x (Online)
Printed in the Philippines

Received: June 28, 2023. Accepted: August 6, 2023
Published online first: February 5, 2024.
https://doi.org/10.15605/jafes.039.01.16

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by a 72-hour fasting test.[5] The presence of consistent symptoms or signs of hypoglycemia accompanied with plasma glucose less than 55 mg/dL (3 mmol/L), insulin at the minimum of 3.0 μIU/mL (18 pmol/L), c-peptide at the minimum of 0.6 ng/ml (0,2 nmol/L), and proinsulin at the minimum of 5.0 pmol/L indicate hyperinsulinemia due to endogenous insulin. β-hydroxybutyrate at most 2.7 mmol/L and elevation in plasma glucose of at least 25 mg/dL after administration of IV glucagon indicate that insulin mediated the hypoglycemia. The coexistence of hypoglycemia and endogenous hyperinsulinemia characterizes insulinoma. Nonetheless, screening for circulating oral hypoglycemic medications and insulin antibodies is crucial in individuals with hypoglycemia and endogenous hyperinsulinemia before diagnosing insulinoma.[6]

Even though the 72-hour fast test yields high efficacy in diagnosing insulinoma qualitatively, this method requires patients to be hospitalized for at least one week. It cannot be performed in an outpatient setting. The induction of hypoglycemia signs and symptoms causes discomfort for the patient. Therefore, further research on more convenient alternative methods of diagnosing insulinoma that could be carried out in an outpatient setting needs to be done. Recent studies have shown measurement of blood components during oral glucose tolerance tests may help diagnose insulinoma.[7,8] Thus, we aimed to evaluate the diagnostic accuracy of oral glucose tolerance test in detecting insulinoma.

METHODOLOGY

This review was reported based on the PRISMA Statement.[9] We published and registered the protocol of this systematic in PROSPERO (CRD42022360205).

Study eligibility

The inclusion criteria that were employed for selecting literature were (1) relevant to the clinical question; (2) a study that includes measurement of any blood component in oral glucose tolerance test as diagnostic tools for diagnosis of insulinoma; (3) a study that includes 72-h fasting test as a reference; and (4) subjects are adult patients with recurrent hypoglycemia episodes.

On the other hand, the exclusion criteria employed for selecting literature were (1) full-text articles are not accessible; (2) study in a language other than English; and (3) studies that include neither sensitivity, specificity, positive predictive value, negative predictive value, nor area under the curve for any blood component measured during oral glucose tolerance test.

Database searches and study selection

The literature search was conducted on 19 August 2022, using several databases such as MEDLINE, Scopus, Embase, and ScienceDirect. The search was done using all relevant medical subheading (MeSH) terms based on the clinical question, including "insulinoma", "72-hour fast", "glucose tolerance test," and their synonyms, as depicted in Table 1. Two investigators (FMC and DLT) independently reviewed the title and abstracts. If any potentially eligible study was identified, the two investigators reviewed the full text of any identified study (FMC and DLT).

Table 1. Literature search query

Databases	Search query	Results
MEDLINE	Keywords: (("Insulinoma") OR ("Insulinomas") OR ("Insulomas") OR ("Insuloma")) AND (("72 hour fasting") OR ("72 hour fast") OR ("72-hour fasting") OR ("72-hour fast") OR ("72-h fast") OR ("72-h fasting")) AND (("glucose tolerance test") OR ("Glucose Tolerance Tests") OR ("glucose tolerance"))	9
Scopus		30
Embase		15
ScienceDirect	Keywords: (("Insulinoma") OR ("Insulinomas")) AND (("72 hour fast") OR ("72-hour fast") OR ("72-h fast") OR ("72-h fasting")) AND (("glucose tolerance test") OR ("Glucose Tolerance Tests")	76

Data extraction

We collected data from each selected study, including study citations, characteristics of included studies, intervention method, and study outcome. Study citations contain the name of the first author, year of publication, and title of the study. Characteristics of selected studies referred to used study design, location, and the number of study participants. The intervention method covered the oral glucose tolerance test used, including any measurement of blood components during OGTT performed in each study. The study outcome included the value of sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, and area under the curve with the respective confidence interval of each model.

Quality assessment and data synthesis

Assessment of included studies was done using the Quality Assessment of Diagnostic Accuracy-2 tool (QUADAS-2) by two independent investigators (FMC and DLT). Qualitative analysis was performed considering the study size, the method of oral glucose tolerance test used, and the value of sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, and area under the curve with its confidence interval.

RESULTS

The flow of the study selection is presented in Figure 1. A total of 130 results were gathered based on a literature search in four scientific databases, in which 23 articles were identified as duplicated. One hundred seven articles were screened through titles and abstracts. Of these, 56 articles were irrelevant to the clinical question, and 45 had the wrong study design. Six articles were left to be

Table 2. Summary of included study characteristics

Author (Year)	Location	Study Design	Population	Method of intervention	Main outcome
Liao et al.[7] (2020)	West China Hospital, China	Case-control	79 patients with recurrent hypoglycemia of which 37 patients had insulinoma and 42 others insulinoma absent from January 2009 to January 2019.	Level of plasma glucose, serum insulin, c-peptide, and HbA1c during 3-h oral glucose tolerance test	2-h/0-h insulin ratio combined with 1-h/0-h c-peptide ratio had high diagnostic accuracy for insulinoma. (Sensitivity 86.5%, Specificity 95.2%, PPV 94.1%, NPV 88.9%, AUC 0.97)
Li et al.[8] (2017)	Sixth People's Hospital Shanghai, China	Case-control	15 patients were diagnosed with insulinoma and 12 patients were diagnosed with reactive hypoglycemia as a control group between December 2009 and December 2014.	Level of plasma glucose, insulin, and c-peptide during 5-h oral glucose tolerance test	5-h Insulin to glucose ratio combined with 0-h c-peptide to glucose ratio had high specificity (83.3%) and sensitivity (100%) for predicting insulinoma. (AUC 0.94)

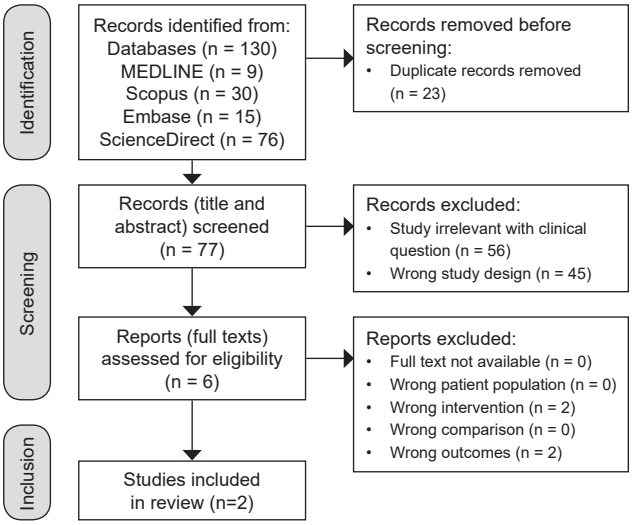

Figure 1. The PRISMA flowchart of search strategy and article selection.

assessed through full text for their eligibility. Two studies were excluded from these articles due to inappropriate intervention and two other studies were excluded due to the inappropriate outcome. Hence, only two studies were left to be further assessed in this systematic review.

The characteristics of the two included studies are shown in Table 2. Both studies were case-control studies with level 4 of evidence based on Oxford Centre for Evidence-Based Medicine: Levels of Evidence 2011. The results of critical appraisal can be found in Table 3 below. Overall judgment was at risk of bias due to unclear risk in both patient selection domains, with low concern regarding applicability.

When compared to the other models, Liao et al.,[7] discovered that the 2-h/0-h insulin ratio and 1-h/0-h C-peptide ratio had the highest diagnostic value while Li et al., found that the combination of insulin-to-glucose ratio at 5-h and C-peptide-to-glucose ratio at 0-h had the highest diagnostic value in their research. Table 4 and Table 5 encompass the main outcome of the study by Liao et al.,[7] and Li et al.,[8] respectively.

Table 3. Quality assessment of included studies using QUADAS-2

Study	Risk of bias				Applicability concerns		
	P	I	R	FT	P	I	R
Liao,[7] 2020	✗	✓	✓	✓	✓	✓	✓
Li,[8] 2017	✗	✓	✓	✓	✓	✓	✓

P = patient selection; I = index test; R = reference standard; FT = flow and timing.
✓ indicates low risk; ✗ indicates high risk; ? indicates unclear risk.

DISCUSSION

Liao et al.,[7] enrolled a total of 79 patients with recurrent hypoglycemia from January 2009 to January 2019 in West China Hospital. They found 37 patients with insulinoma, of those three patients were diagnosed clinically, while the 34 others were diagnosed pathologically. On the other hand, 42 patients with hypoglycemia due to other causes such as liver damage, endosecretory diseases, and paraneoplastic syndrome, were categorized into the control group. Both groups were significantly distinct in terms of duration of hospitalization, HbA1c, fasting glucose, insulin, and C-peptide level (p <0.05). In contrast, the age and sex of the patients between them were not significantly different (p >0.05).

The main outcome of this study was the 2-h/0-h insulin ratio combined with the 1-h/0-h C-peptide ratio taken during 3-h OGTT had the highest diagnostic value in the diagnosis of insulinoma, which had the largest AUC (0.97; 95% CI 0.90–0.99) in this study. The sensitivity and specificity were 86.5% (95% CI 71.2–95.5%) and 95.2% (95% CI 83.8–99.4%), respectively. 2-h/0-h insulin ratio combined with 1-h/0-h C-peptide ratio was calculated from the logistic regression model as 8.305 – (0.441 × insulin 2-h/0-h) – (1.679 × C-peptide 1-h/0-h), with the cut-off value of >0.351. A score greater than 0.351 indicates the diagnosis of insulinoma and vice versa. The positive predictive value of 2-h/0-h insulin ratio + 1-h/0-h C-peptide ratio was 94.1% (95% CI 80.4-98.4%). This value means that of 100 people diagnosed with insulinoma using this method, only 5.9 were falsely accused of insulinoma. The negative predictive value of 2-h/0-h insulin ratio + 1-h/0-h C-peptide ratio was 88.9 (95% CI 77.9–94.8%), which explains that in 100 people tested for negative results using this method, 11.1 of them were had an insulinoma.[7]

One of the advantages of their study was the comparison of the diagnostic value between their model and the 72-h fast test as the reference standard. The sensitivity and

Table 4. Main outcome of the study by Liao et al.[7]

Results	Insulin 2-h/0-h + C-peptide 1-h/0-h* >0.351 (95% CI)	C-peptide 1-h/0-h + HbA1c > −0.142 (95% CI)	C-peptide 1-h/0-h + Glucose 1-h >0.554 (95% CI)	C-peptide 1-h/0-h + Glucose 0-h > −0.333 (95% CI)	C-peptide 1-h/0-h ≤3.582 (95% CI)	Insulin 2-h/0-h + Glucose 0-h >0.258 (95% CI)
Sensitivity	86.5 (71.2–95.5)	94.6 (81.8–99.3)	83.8 (68.0–93.8)	89.2 (74.6–97.0)	89.2 (74.6–97.0)	78.4 (61.8–90.2)
Specificity	95.2 (83.8–99.4)	90.5 (77.4–97.3)	92.9 (80.5–98.5)	88.1 (74.4–96.0)	85.7 (71.5–94.6)	90.5 (77.4–97.3)
Positive predictive value	94.1(80.4–98.4)	89.7 (77.4–95.7)	91.2 (77.5–96.9)	86.8 (74.2–93.8)	84.6 (72.2–92.1)	87.9 (73.8–94.9)
Negative predictive value	88.9 (77.9–94.8)	95.0 (83.1–98.7)	86.7 (75.7–93.1)	90.2 (78.5–95.9)	90.0 (78.0–95.8)	82.6 (71.8–89.8)
Positive likelihood ratio	18.2 (4.7–70.7)	9.9 (3.9–25.3)	11.7 (3.9–35.2)	7.5 (3.3–17.2)	6.2 (3.0–13.2)	8.2 (3.2–21.2)
Negative likelihood ratio	0.1 (0.1–0.3)	0.1 (0.0–0.2)	0.2 (0.1–0.4)	0.1 (0.1–0.3)	0.1 (0.1–0.3)	0.2 (0.1–0.4)
Area under the curve	0.97 (0.90–0.99)	0.95 (0.87–0.98)	0.94 (0.86–0.98)	0.92 (0.84–0.97)	0.91 (0.82–0.96)	0.89 (0.80–0.95)

*Insulin 2-h/0-h + C-peptide 1-h/0-h was calculated as: 8.305 – (0.441 × insulin 2 h/0 h) – (1.679 × C-peptide 1 h/0 h)

Table 5. Main outcome of the study by Li et al.[8]

Results	Insulin-to-glucose ratio* 5-h >20.45 pmol/mmol + C-peptide-to-glucose ratio† 0-h <0.19 nmol/mmol (95% CI)	Insulin-to-glucose ratio* 5-h >20.45 pmol/mmol + Insulin-to-glucose ratio* 0-h >13.54 pmol/mmol (95% CI)	Insulin-to-glucose ratio* 5-h >20.45 pmol/mmol (95% CI)	Insulin-to-glucose ratio* 0-h >13.54 pmol/mmol (95% CI)	C-peptide-to-glucose ratio† 5-h (95% CI)	C-peptide-to-glucose ratio† 0-h <0.19 nmol/mmol (95% CI)
Sensitivity	100 (78.0–100)	93.33 (68.0–98.9)	80.0 (51.9–95.4)	93.33 (68.0–98.9)	86.67 (59.5–98.0)	73.33 (44.9–92.0)
Specificity	83.3 (51.6–97.4)	83.33 (51.6–97.4)	91.67 (61.5–98.6)	75.0 (42.8–94.2)	75.0 (42.8–94.2)	83.33 (51.6–97.4)
Positive predictive value	88.2 (63.5–98.2)	87.5 (61.6–98.1)	92.3 (63.9–8.7)	82.4 (56.6–96.0)	81.2 (54.3–95.7)	84.6 (54.5–97.6)
Negative predictive value	100 (69-100)	90.9 (58.7–98.5)	78.6 (49.2–95.1)	90.0 (55.5–98.3)	81.8 (48.2–97.2)	71.4 (41.9–91.4)
Positive likelihood ratio	6.0 (4.7–7.7)	5.6 (4.2–7.5)	9.6 (7.1–13.0)	3.73 (2.6–5.3)	3.47 (2.4– 5.1)	4.4 (3.0–6.5)
Negative likelihood ratio	0.00 (N/A)	0.08 (0.01–0.8)	0.22 (0.03– 1.8)	0.09 (0.01–0.7)	0.18 (0.04–0.9)	0.32 (0.07–1.5)
Area under the curve	0.94 (0.78–1.00)	0.94 (0.78–1.00)	0.91 (0.74–0.99)	0.87 (0.68–0.97)	0.82 (0.62–0.94)	0.84 (0.65–0.95)

N/A = not applicable
*Insulin-to-glucose ratio = insulin (pmol/L)/glucose (mmol/L)
†C-peptide-to-glucose ratio = C-peptide (nmol/L)/glucose (mmol/L)

specificity of the 72-h fast test, described as blood glucose <3 mmol/L, insulin >3 μIU/ml, and C-peptide >0.2 nmol/l measured after 10-h overnight prolonged fasting had high specificity but low sensitivity in diagnosing insulinoma. The sensitivity and specificity were 88.1% (95% CI 75.0–94.8%) and 43.2% (95% CI 28.7–59.1%), respectively. A total of 16 (43.2%) subjects from the insulinoma group and 5 (11.9%) subjects from the control group had positive results using the 72-hour fast test. While using Liao et al., model, they yielded a total of 32 (86.5%) subjects with positive results in the insulinoma group, and only two (4.8%) subjects in the control group had positive results. Hence, compared to the reference standard, the 2-h/0-h insulin ratio + 1-h/0-h C-peptide ratio had higher diagnostic accuracy in diagnosing insulinoma.[7]

In the study conducted by Li et al.,[8] a total of 15 patients with the diagnosis of insulinoma and 12 patients with diagnosis of reactive hypoglycemia as control group were enrolled. This study covered all patients with insulinoma and reactive hypoglycemia in Sixth People's Hospital between December 2009 and December 2014. Patients in the insulinoma group had significantly higher BMI but lower total bilirubin, high-density lipoprotein cholesterol (HDL), glycated albumin, and HbA1c than patients in the reactive hypoglycemia group.

This study concluded that the combination of insulin-to-glucose ratio at 5-h higher than 20.45 pmol/mmol and C-peptide-to-glucose ratio at 0-h lower than 0.19 nmol/mmol had the highest specificity (83.3%; 95% CI 51.6%-97.4%) and sensitivity (100%; 95% CI 78-100%) in diagnosing insulinoma with highest AUC of 0.94 (95% CI 0.78-1.00). The positive predictive value of this method was 88.2% (95% CI 63.5-98.2%), and the negative predictive value was 100% (95% CI 69-100%). Insulin-to-glucose ratio was calculated as insulin (pmol/L)/glucose (mmol/L), and the C-peptide-to-glucose ratio was calculated as C-peptide (nmol/L)/glucose (mmol/L).[8]

Li et al.,[8] then implemented their new model to screen for insulinoma in 75 patients with a primary complaint of hypoglycemia during the initial visit. All patients underwent 5-h OGTT. The diagnostic value of insulin-to-glucose ratio at 5-h + C-peptide-to-glucose ratio at 0-h in this population were 82.67% for sensitivity, 73.08% for specificity, 57.58% for positive predictive value, and 90.48% for negative predictive value.

In conclusion, the two studies conducted by Liao et al.,[7] and Li et al.,[8] discovered that using OGTT may be a novel approach with good diagnostic value for identifying insulinoma in patients with hypoglycemia. The gold standard 72-hour fasting test requires hospitalization and careful monitoring, which is time-consuming, inconvenient, and unpleasant for the patients. The OGTT provides an easier approach that may be performed in a single outpatient visit. Although Liao et al.,[7] and Li et al.,[8] had different indicators and cut-off values in the diagnosis of insulinoma, both studies had a similar method which measured plasma glucose, serum insulin, and C-peptide level from blood samples taken at 0-h, 1-h, 2-h, and 3-h in Liao et al.[7] study and additional of 4-h and 5-h blood samples in Li et al.[8] study. For the best convenience and

cost-effectiveness, we recommend the 2-h OGTT test after 10 hours of overnight fasting with measurement of insulin and C-peptide during 0-h and 2-h in recurrent hypoglycemia patients suspected of insulinoma. Also, the 2-h OGTT is less expensive than the standard 72-hour fasting test, which requires extensive hospitalization.

Nonetheless, there are several limitations in this systematic review. First, since insulinoma is a rare cause of hypoglycemia, only limited research in OGTT as an alternative to diagnosing insulinoma is available to this date. In this area of research, there were no systematic reviews and meta-analyses available during the literature search. We only included two low-level evidence studies at risk of bias due to patient selection bias. Second, all the studies included in this systematic review were conducted in a small sample size population within the Chinese population. Third, quantitative analysis could not be performed in our systematic review due to a limited number of studies and outcome variety between studies. Additional research with a large sample size is required to validate the established model before implementing OGTT as a diagnostic tool in diagnosing insulinoma.

CONCLUSIONS

The measurement of 0-h and 2-h insulin and C-peptide levels during 2-h OGTT was found in two small case-control studies with a total of 106 patients to have high diagnostic values. However, due to these limitations, future research is still needed to validate the potential use of OGTT for the diagnosis of insulinoma.

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

CRediT Author Statement
FC: Conceptualization, Methodology, Software, Formal analysis, Investigation Resources, Data curation, Writing – original draft preparation, Visualization, Project administration; **DT:** Conceptualization, Methodology, Validation, Review and editing, Supervision, Funding acquisition.

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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Journal of the ASEAN Federation of Endocrine Societies

Review Article

The Roles of Non-Pharmacologic and Emerging Pharmacologic Management of Non-alcoholic Fatty Liver Disease and Sarcopenia: A Narrative Review

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is one of the most prevalent causes of chronic liver disease worldwide which is often seen in patients with metabolic abnormalities such as those with obesity and insulin resistance. On the other hand, sarcopenia is a generalized and progressive skeletal muscle disorder characterized by low muscle strength, low muscle quality, low physical performance, or a combination of the three. Both disease entities share several underlying risk factors and pathophysiologic mechanisms. These include: (1) cardiometabolic overlaps such as insulin resistance, chronic systemic inflammation, decreased vitamin D levels, sex hormone modifications; (2) muscle-related factors such as those mitigated by myostatin signaling, and myokines (i.e., irisin); and (3) liver-dysfunction related factors such as those associated with growth hormone/insulin-like growth factor 1 Axis, hepatokines (i.e., selenoprotein P and leukocyte cell-derived chemotaxin-2), fibroblast growth factors 21 and 19 (FGF21 and FGF19), and hyperammonemia. This narrative review will examine the pathophysiologic overlaps that can explain the links between NAFLD and sarcopenia. Furthermore, this review will explore the emerging roles of nonpharmacologic (e.g., weight reduction, diet, alcohol, and smoking cessation, and physical activity) and pharmacologic management (e.g., roles of β-hydroxy-β-methylbutyrate, branched-chain amino acid supplements, and testosterone therapy) to improve care, intervention sustainability, and acceptability for patients with sarcopenia-associated NAFLD.

Key words: NAFLD, sarcopenia, fatty liver disease

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease often seen in individuals with metabolic abnormalities (e.g., obesity and insulin resistance). Sarcopenia is a condition characterized by the loss of muscle mass and strength that typically occurs with aging and obesity. Together, sarcopenia and NAFLD share many underlying risk factors and pathophysiologic mechanisms, which are primarily addressed by lifestyle interventions such as alcohol cessation, physical activity, and a hypocaloric diet. Through this descriptive study, we aim to further discuss the clinical associations and pathophysiologic mechanisms that link sarcopenia and NAFLD, then focus on the role of nonpharmacologic management and emerging pharmacologic options in NAFLD-associated sarcopenia.

Non-alcoholic fatty liver disease (NAFLD)

Non-alcoholic fatty liver disease is one of the most important causes of liver diseases worldwide and is likely to emerge as the leading cause of end-stage liver disease in the future.[1] In the United States of America (USA), an estimated 80-100 million individuals are affected by NAFLD, and with the spread of obesity worldwide, there has also been a concurrent rise in the prevalence of NAFLD in both developed and developing countries.[1,2]

According to the American Association for the Study of Liver Diseases (AASLD), NAFLD is defined by (1) evidence of hepatic steatosis [i.e., by imaging or histology], and (2) the lack of secondary causes of hepatic fat accumulation [e.g., significant alcohol consumption, the use of steatogenic medication, or monogenic hereditary disorders].[3]

eISSN 2308-118x (Online)
Printed in the Philippines

Received: March 28, 2023. Accepted: May 30, 2023.
Published online first: October 27, 2023.
https://doi.org/10.15605/jafes.039.01.04

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Metabolic comorbidities such as type 2 diabetes mellitus (T2DM), dyslipidemia, and obesity are strongly linked with the development of NAFLD.[4] Studies have shown that NAFLD is increasingly recognized as the hepatic manifestation of metabolic syndrome.[1,2] Furthermore, NAFLD itself is a risk factor for cardiovascular disease (CVD), chronic kidney disease (CKD), and T2DM.[4]

Sarcopenia

Sarcopenia is defined by the European Working Group on Sarcopenia in Older People (EWGSOP) as a generalized and progressive skeletal muscle disorder characterized by low muscle strength, low muscle quality, low physical performance, or a combination of the three.[5] A wide variety of tests and tools are available to diagnose sarcopenia, which utilize the above-mentioned EWGSOP criteria to identify the condition. Muscle mass can be measured with magnetic resonance imaging (MRI) and CT scanning, dual x-ray absorptiometry (DXA), ultrasound, bioimpedance analysis, calf circumference measurement, and D3-creatine dilution.[6] Muscle strength is assessed with handgrip strength using a handheld hydraulic dynamometer or knee extensor force, while physical performance can be measured with gait speed, the Short Physical Performance Battery (SPPB) test, and the Timed Up and Go (TUG) test.[7-9] Sarcopenia has varying definitions and consequently varying approaches to diagnosis, which inevitably affects its estimated prevalence.[10] A recent meta-analysis by Petermann-Rocha et al. found that the prevalence of sarcopenia ranges from 10% to 27% in individuals over 60 years old, noting that the values vary considerably according to the classification and cut-off point used.[11] Other definitions for sarcopenia include those developed by the International Working Group on Sarcopenia (IWGS) and the Asian Working Group on Sarcopenia (AWGS).[12,13] The EWGSOP2, IWGS, and AWGS 2019 adopt similar approaches in defining sarcopenia based on low muscle mass in combination with poor muscle function. Cut-off points for Asian populations were lower compared to non-Asian populations in the previous AWGS 2014 definition but have since been revised in the AWGS 2019 definition of sarcopenia (Table 1).[13,14]

Link between NAFLD and sarcopenia

Sarcopenia and NAFLD share many common underlying pathophysiologic mechanisms, such as insulin resistance, chronic inflammation, nutritional deficiencies, and physical inactivity.[15,16] Both are associated with obesity, metabolic syndrome, type 2 diabetes, dyslipidemia, and age.[17,18] In a recent population study by Hong et al., low levels of serum vitamin D (i.e. serum 25(OH)D level of 4.85–15.26 ng/mL) were found to be associated with sarcopenia (OR 2.65; 95% CI 1.64–4.27 in males and OR 1.80; 95% CI 1.29–2.51 in females], NAFLD (OR 1.82; 95% CI 1.19–2.96 in males), and sarcopenia in NAFLD (OR 2.25; 95% CI 1.26–4.03 in males).[19] Low muscle mass and low muscle strength are also positively associated with NAFLD, and concurrent sarcopenia and obesity are associated with a significantly higher risk of NAFLD.[20,21] A prospective study of UK Biobank participants identifies low muscle mass and grip strength as risk factors associated with a higher risk of severe NAFLD.[11] The prevalence of sarcopenia increases with age and is closely associated with obesity, especially for older adults.[22] In American populations, the prevalence of sarcopenia varies across race and ethnicity, with Hispanics having the highest prevalence, followed by Asians, non-Hispanic Whites, and lastly non-Hispanic Blacks.[22,23]

Moreover, multiple population studies have demonstrated the association between NAFLD and sarcopenia.[24-27] Several of these recent studies are summarized in Table 2. It must be noted that most of these studies were conducted in Asian populations, and the methods of assessing and defining NAFLD and sarcopenia differ as well.

METHODOLOGY

This narrative review focused on the latest literature on the interrelationship between non-alcoholic fatty liver disease and sarcopenia, the majority dating from 2015-2023. Studies included in this review were done using SCOPUS, MEDLINE, and CINAHL databases. Search strings included 'Non-Alcoholic Fatty Liver Disease' OR 'NAFLD and 'Sarcopenia.' Additionally, reference lists of primary articles identified through initial searches and manual searches for further pertinent journals were carried out by the researchers.

PATHOPHYSIOLOGIC MECHANISMS EXPLAINING THE LINK BETWEEN NAFLD AND SARCOPENIA

Cardiometabolic overlaps between NAFLD and sarcopenia

The pathophysiologic processes behind sarcopenia and NAFLD are multifactorial. Recent studies have shown

Table 1. Definition of Sarcopenia developed by the European, International, and Asian Working Groups on Sarcopenia

	Low muscle mass	Low muscle strength (grip strength)	Low muscle performance (gait speed, short performance physical battery)
EWGSOP2	Males: <7.0 kg/m^2 Females: <5.5 kg/m^2	Males: <27 kg Females: <16 kg	Gait speed ≤0.8 m/s SPPB ≤8-point score
IWGS	Males: <7.23 kg/m^2 Females: <5.67 kg/m^2	–	Gait speed ≤1.0 m/s
AWGS 2019	Males: <7.0 kg/m^2 Females: <5.4 kg/m^2	Males: <28 kg Females: <18 kg	Gait speed ≤1.0 m/s SPPB ≤9-point score

Adapted from Cruz-Jentoft et al., 2019; Fielding et al., 2011; Chen et al., 2020
Abbreviations: AWGS = Asian Working Group on Sarcopenia; EWGSOP = European Working Group on Sarcopenia in Older People; IWGS = International Working Group on Sarcopenia

that sarcopenia and NAFLD share several common pathophysiologic mechanisms. Figure 1 illustrates the key mechanisms linking sarcopenia and NAFLD.

Insulin resistance

Skeletal muscles account for nearly 50% of lean body mass. Glucose uptake in the skeletal muscle is essentially insulin-dependent, occurring via glucose transporter 4. Thus, skeletal muscles play an integral role in glucose and energy homeostasis.

Insulin plays an important role in protein synthesis, proteolysis inhibition, amino acid transport in skeletal muscle, and muscle proliferation and hypertrophy.[28] Insulin normally increases the activity of phosphatidylinositol 3-kinase (PI3K)/Akt signaling which phosphorylates Forkhead box O (FoxO) transcription factors inhibiting its activation and preventing the induction of atrophy-related muscle-specific ubiquitin ligases: atrogin-1 and muscle ring finger 1 protein (Murf1), that promote skeletal muscle atrophy. Hence, insulin resistance (IR) is an established risk factor for both NAFLD and sarcopenia.[29,30]

In a state of IR, the insulin-mediated degradation of FoxO transcription factors via the PI3K/Akt pathway is ineffective resulting in its accumulation in the nucleus and induction of the atrophy-related ligases causing skeletal muscle atrophy.[29,30] Furthermore, cells fail to respond normally to insulin becoming less effective in taking up glucose from the blood, thereby worsening sarcopenia. Hence, insulin resistance can lead to 1) a reduction of protein synthesis; 2) the promotion of proteolysis and muscle wasting, and 3) the promotion of lipolysis and increased circulating fatty acids (FA) that can be taken up by both muscle and liver. Excess FA stored within the muscle results in myosteatosis (i.e., further reducing muscle protein synthesis which

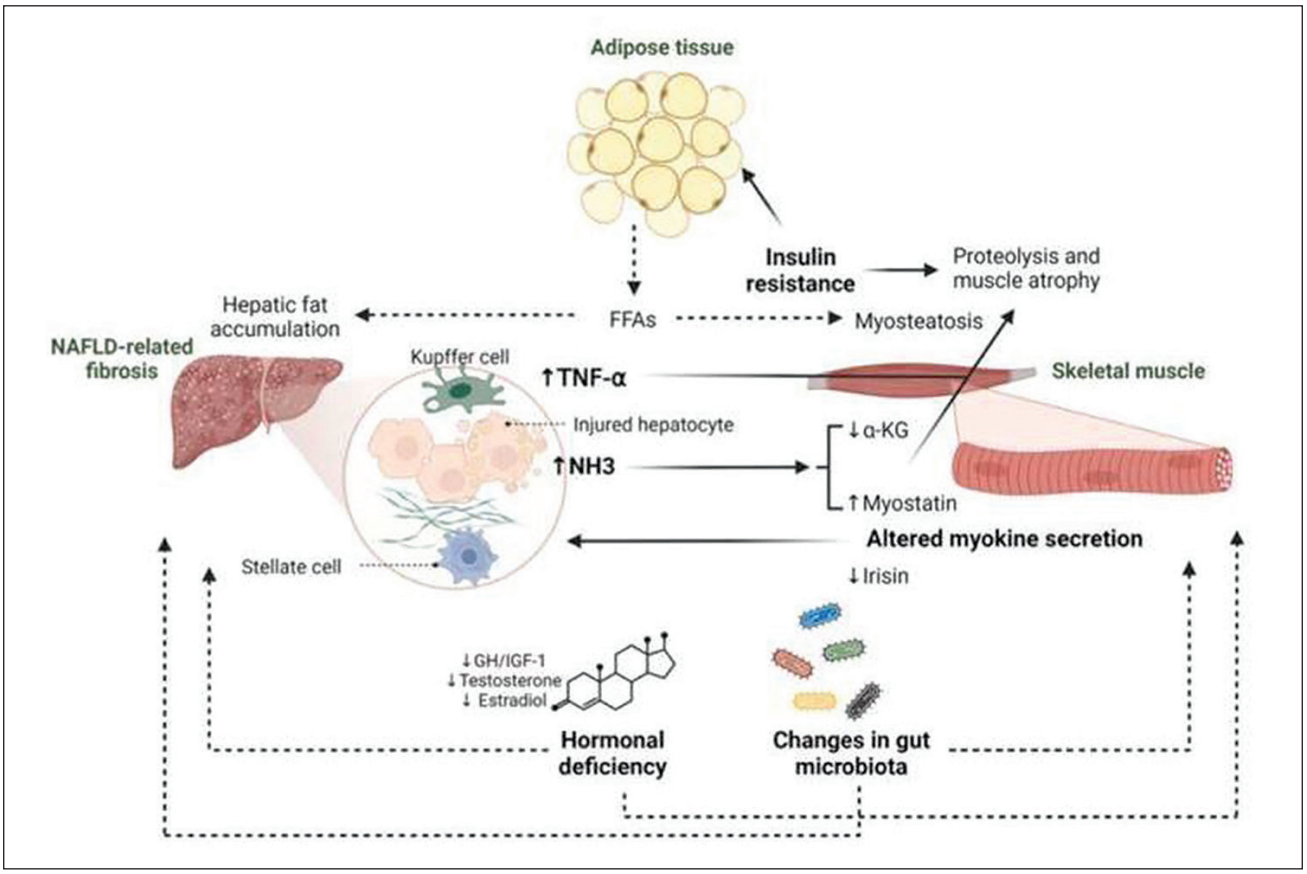

Figure 1. Pathophysiologic mechanisms involved in the relationship between NAFLD and sarcopenia.

Adapted from "Non-alcoholic fatty liver disease-related fibrosis and sarcopenia: An altered liver-muscle crosstalk leading to increased mortality risk," by Kuchay, M. et al., 2022, Ageing Research Reviews, 80. Copyright [2022] by Elsevier B.V.

Table 2. Population studies that link sarcopenia and NAFLD

Study	Population (N)	Key Findings
Roh et al. (2022)	Korea (1595)	Those with NAFLD have 1.6x risk of developing low muscle mass (LMM) and 2.29x risk of developing low muscle strength (LMS)
Wang et al. (2021)	China (578)	LMM independently associated with NAFLD in both males and females; LMS independently associated with NAFLD in males only
Wijarnpreecha et al. (2021)	United States (1925)	Individuals with sarcopenic obesity have significantly increased odds of having NAFLD
Song et al. (2022)	Korea (2191)	Prevalence of NAFLD and fibrosis increased significantly according to sarcopenic status
Chung et al. (2019)	Korea (5989)	The prevalence of NAFLD was significantly higher in subjects with sarcopenia than in those without

contributes to sarcopenia), while increased delivery of free fatty acids to the liver promotes lipotoxicity that can worsen NAFLD.[30,31] Sarcopenia, on the other hand, can enhance IR by reducing cellular targets for insulin action.

Chronic systemic inflammation

NAFLD has been recognized as a subclinical inflammatory state, characterized by the activation of Kupffer cells and subsequent production of tumor necrosis factor-alpha (TNF-α) as well as other pro-inflammatory cytokines that influence disease progression. In an advanced disease state, TNF-α produced by a fibrotic liver can be transported to the skeletal muscle and induce muscle atrophy through the activation of nuclear factor kappa B (NF-kB) resulting in the upregulation of Muscle ring finger 1 (Murf1) protein which mediates sarcomeric protein degradation causing skeletal muscle atrophy.[30] Thus, targeting TNF-α inhibition in skeletal muscles may be a potential preventive strategy for liver-fibrosis-induced muscle atrophy. Other proinflammatory cytokines (e.g., Interleukin (IL)-6 and IL-β) can also promote apoptosis in skeletal muscles. This is seen in chronic inflammatory states such as in NAFLD wherein IL-6 inhibits IGF-1 activity, reducing myogenesis.[28]

Decreased vitamin D levels

Vitamin D is integral in myoblast proliferation and differentiation, skeletal muscle growth, and reduction of muscle inflammation. Furthermore, it regulates the expression of insulin receptors in pancreatic β-cells and peripheral target tissues. Receptors of vitamin D are expressed in cells including the liver and skeletal muscles. Hence, patients with NAFLD have been found to have lower levels of vitamin D leading to worsening inflammation and the promotion of liver fibrosis.[28]

Sex hormones

Testosterone is an anabolic hormone that promotes muscle protein synthesis, and insulin sensitivity, and decreases fat mass. In men, testosterone deficiency has been associated with an increased accumulation of visceral adipose tissue, insulin resistance, and subsequently increased transport of free fatty acids to the liver, all of which contribute to the development and progression of NAFLD.[32] Age-related decrease in sex hormone levels (i.e., testosterone and estrogen deficiency in males and females, respectively) has been associated with sarcopenia, sarcopenic obesity, and its related complications, such as NAFLD. These may explain the higher rates of sarcopenia and NAFLD in elderly patients.[30]

Muscle-related factors that influence NAFLD progression

Myostatin signaling

Myostatin is a myokine synthesized and secreted in skeletal muscle which plays an integral role in the inhibition of skeletal muscle growth and mass. It works by binding to the activin receptor type IIB (ActRIIB) resulting in the formation and activation of the Smad complex that inhibits muscle hypertrophy and hyperplasia as well as suppresses protein synthesis. Elevated levels of myostatin have been reported in patients with liver fibrosis and cirrhosis.[28,30] Myostatin worsens liver fibrosis through the activation of ActRIIB on hepatic stellate cells reducing stellate cell proliferation, inducing cell migration, and increasing procollagen type 1, a biomarker associated with liver injury, inflammation, and fibrosis.[30]

Irisin

Decreased skeletal muscle as in sarcopenia reduces the secretion of various beneficial myokines such as irisin, which is another myokine that acts on the skeletal muscle, influences glucose homeostasis, increases adipocyte energy expenditure, and improves insulin sensitivity and lipid metabolism. Sarcopenia has also been associated with a reduction of pro-inflammatory cytokines and an elevation of anti-inflammatory cytokines in adipose tissue. Irisin's ability to regulate lipid metabolism which may help prevent lipid accumulation in liver cells is another potential mechanism that may explain the link between sarcopenia in NAFLD.[28,33] Irisin secretion is induced by exercise which may support the link between physical inactivity and NAFLD.[34]

Liver dysfunction-related factors that promote sarcopenia

Growth hormone/insulin-like growth factor 1 axis

Growth hormone (GH) acts on insulin-sensitive organs (e.g. liver, adipose tissue, and skeletal muscle). Its effects are mediated by insulin-like growth factor-1 (IGF-1) synthesized in the liver. Together, they are key regulators of metabolic homeostasis. It has been demonstrated that patients with NAFLD have reduced IGF-1 gene expression.[35] With suppression of the GH/IGF-1 axis activity, Akt is dephosphorylated resulting in the loss of inhibition of FoxO transcription factors and subsequent increased expression of muscle-specific ubiquitin ligases, atrogin-1, and Murf, thus, promoting skeletal muscle atrophy.[30] Decreased levels of GH and IGF-1 have also been observed in obesity and in aging which may explain their relationship with NAFLD and sarcopenia.

Hepatokines (Selenoprotein P and leukocyte cell-derived chemotaxin-2)

Non-alcoholic fatty liver disease induces the production of oxygen free radicals which results in lipid peroxidation and the production of proinflammatory cytokines, such as TNF-α and TGF-β. Several hepatokines act in an auto-, para-, and endocrine manner to regulate a broad range of metabolic processes. Selenoprotein P (SeP), a hepatokine that works as a selenium transport protein, is increased in NAFLD as well as in type 2 diabetes and pre-diabetes.[29,31] SeP inhibits 5'AMP-activated protein kinase (AMPK) in the liver promoting the synthesis of leukocyte cell-derived chemotaxin-2 (LECT2), which induces IR in skeletal muscle. Both SeP and LECT2 provide a direct association between NAFLD and muscle dysfunction.[29]

Table 3. Screening for Sarcopenia: SARC-F Scoring, 5- and 7-item MSRA

SARC-F		MRSA	7	5
Strength: How much difficulty do you have in lifting and carrying 10 lb?	None = 0 Some = 1 A lot or unable = 2	1. How old are you?		
		• ≥70 years	0	0
		• <70 years	5	5
Assitance in walking: How much difficulty do you have walking across a room?	None = 0 Some = 1 A lot, use aids, or unable = 2	2. Were you hospitalized in the last year?		
		• Yes, ≥1	0	0
		• Yes, 1	5	10
		• No	10	15
Rise from a chair: How much difficulty do you have transferring from a chair or bed?	None = 0 Some = 1 A lot or unable without help = 2	3. What is your activity level?		
		• Able to walk <1000 m	0	0
		• Able to walk >1000 m	5	15
Climb stairs: How much difficulty do you have climbing a flight of 10 stairs?	None = 0 Some = 1 A lot or unable = 2	4. Do you eat 3 meals per day regularly?		
		• No, up to twice per week I skip a meal (for example, I skip breakfast or I have only milky coffee or soup for dinner)	0	0
		• Yes	5	15
Falls: How many times have you fallen in the past year?	None = 0 1–3 falls = 1 ≥ 4 falls = 2	5. Do you consume any of the following? Milk or dairy products (yogurt, cheese)		
		• Not every day	0	-
		• At least once per day	5	-
		6. Do you consume any of the following? Poultry, meat, fish, eggs, legumes, ragout, or ham		
		• Not every day	0	-
		• At least once per day	5	-
		7. Did you lose weight in the past year?		
		• >2 kg	0	0
		• ≤2 kg	5	10

Abbreviations: SARC-F: Strength, Assistance with walking, Rise from a chair, Climb stairs and Falls; score ≥4 is predictive of sarcopenia.
MSRA: Mini Sarcopenia Risk Assessment; 5-item ≤45 and 7-item ≤30 are indicative of risk of sarcopenia.

Table 4. Summary of key findings

Non-alcoholic fatty liver disease increases the risk of developing low muscle mass and low muscle strength, commonly manifesting as sarcopenic obesity.
Overlaps in pathophysiology between NAFLD and sarcopenia are related to insulin resistance, chronic systemic inflammation, decreased vitamin D levels, and sex hormones.
Screening for sarcopenia in NAFLD patients using the SARC-F Scoring and 7-item MSRA are crucial to prevent the progression of sarcopenia.
Weight reduction through an individualized hypocaloric Mediterranean Diet targeting 1200–1500 kcal/d or a reduction of 500–1000 kcal/d from baseline and a target protein intake of 1.2–1.5 g/kg/day is recommended.
In NAFLD alone, vigorous-intensity aerobic exercise is recommended. However, in NAFLD-associated sarcopenia, supervised resistance and hypertrophic training are recommended to revert muscular mass loss.
Emergent treatment options includes Beta-hydroxy-beta-methyl butyrate, branched-chain amino acid supplements, and testosterone therapy.

Abbreviations: NAFLD = Non-alcoholic fatty liver disease; SARC-F = Strength, Assistance with walking, Rise from a chair, Climb stairs and Falls; MSRA = Mini Sarcopenia Risk Assessment

Fibroblast growth factors 21 and 19 (FGF21 and FGF19)

FGF21 is synthesized in the liver, pancreas, adipose tissue, and muscle and plays a role in several mechanisms. In the liver, it plays a protective role by inhibiting liver lipogenesis, stimulating hepatic fatty acid oxidation, and reducing lipid transport into the liver by increasing peripheral lipoprotein catabolism and reducing adipocyte lipolysis.[36] FGF21 enhances insulin sensitivity in both adipose tissue and skeletal muscle which further enhances glucose utilization. Elevated levels of FGF21 have been observed in patients with NAFLD and other disease processes such as non-alcoholic steatohepatitis, obesity, type 2 diabetes, and chronic kidney disease, among others. However, despite this elevation, FGF21 resistance is hypothesized to occur in these disease states.[37] FGF19 also exerts its action in skeletal muscles by inducing muscle hypertrophy and increasing muscle strength. Dysregulation of endocrine FGF signaling, particularly FGF21, and FGF19, has been observed in NAFLD and may help explain its association with sarcopenia.[29,37]

Hyperammonemia

The liver is primarily involved in physiologic ammonia disposal. However, ureagenesis becomes ineffective in diseases that impair liver function. NAFLD is characterized by urea-cycle dysregulation resulting in hyperammonemia. Two mechanisms have been proposed to explain how hyperammonemia promotes sarcopenia. The first one is due to the increased expression of NF-kB and myostatin. The second mechanism is due to the shift into non-hepatocyte ammonia disposal in skeletal muscles in patients with impaired liver function. Ammonia disposal in skeletal muscles results in the loss of α-keto- glutarate (α-KG), a critical tricarboxylic acid (TCA) cycle intermediate, that promotes skeletal muscle protein synthesis and muscle hypertrophy, as well as inhibits protein degradation.[30,38] Hyperammonemia can also promote disease progression by promoting liver fibrosis mainly through the activation of hepatic stellate cells.

DIAGNOSIS

The pioneer in community screening for sarcopenia is the SARC-F questionnaire (Table 3), which is composed of the following domains: Strength, Assistance with walking, Rising from a chair, Climbing stairs, and Falls. Out of 10, a score ≥4 is predictive of sarcopenia. This tool was validated against 3 international consensus panel sarcopenia criteria: European Working Group on Sarcopenia in Older People (EWGSOP), International Working Group on Sarcopenia

(IWGS), and AWGS, resulting in a specificity of 94–99% but poor sensitivity of 4–10%.[39] Similarly, in a meta-analysis composed of 12,800 eligible subjects, the pooled results of SARC-F specificity and sensitivity were 90% and 21%, respectively using the EWGSOP criteria.[40] However, in a recent study on the geriatric population, the ideal SARC-F cut-off was adjusted to 3 when the Youden index was applied, resulting in a specificity of 77% and a sensitivity of 52%.[41] In addition, the prognostic utility of SARC-F was studied in primary six racial/ethnic groups (African Americans, Latinos, Japanese, Native Hawaiians, Whites, and other Asian Americans including Filipinos, Chinese, and Koreans), showing statistically significant association with risk of all-cause mortality in males and females, with adjusted HR for overall mortality of 3.73 and 3.10, respectively.[40] Due to the low sensitivity demonstrated by SARC-F, Rossi et al. developed the Mini Sarcopenia Risk Assessment (MSRA) questionnaire in two forms, the 5- and 7-items (shown in Table 3), wherein scores of ≤45 and ≤30 are indicative of sarcopenia risk, respectively.[42] Against the AWGS criteria, the specificity and sensitivity of MSRA-7 and MRSA-5 were 40%, 87%, 71%, and 90%, respectively.[43] Meanwhile, the combination of SARC-F and 7-item MSRA resulted in a specificity of 100% and sensitivity of 63%; hence, this is recommended as a first-line tool for high-risk patients (Table 3).[41] In a multicenter, retrospective study involving 23,889 NAFLD patients, a high-risk sarcopenic obesity (SO) screening model was developed to identify high-risk SO subjects. Based on the model showing older age, male, sarcopenia index, and metabolic syndrome as significant risk factors, high-risk SO subjects had significantly higher odds of significant liver fibrosis (fibrosis-4 index >2.67) or atherosclerotic CVD risk score >20% compared to subjects without SO.[44] Early identification of sarcopenia before the onset of physical disability is crucial to prevent the progression of sarcopenia.

In patients with chronic liver disease, a cross-sectional study with 661 patients reported the usefulness of arm and calf circumferences, which are correlated with skeletal muscle index and grip strength, as simple surrogate markers for detecting sarcopenia. The optimal cut-off values of arm and calf circumferences were respectively determined to be 25.0 and 32.6 cm for males, and 22.7 and 32.1 cm for females.[45] Recently, the lowest quintile of serum 25(OH)D level (4.85–15.26 ng/mL) was associated with increased occurrence of sarcopenia for both males and females, NAFLD in males, and sarcopenia-associated NAFLD in males but not in females. Hence, Vitamin D levels can also be a useful marker for sarcopenia and NAFLD, especially in males.[46]

MANAGEMENT

Specifically, for use in primary health care and community health promotion, the Asian Working Group for Sarcopenia (AWGS) 2019 introduced the concept of "possible sarcopenia," defined as low muscle strength regardless of reduced physical performance. This aims to increase awareness of sarcopenia prevention through timely lifestyle interventions and to encourage referral to the hospital for confirmatory diagnosis.[13] In this narrative review, we will focus on non-pharmacologic management and prevention, which remains the cornerstone of sarcopenia-associated NAFLD treatment.

Non-pharmacological management

Weight Reduction

Sarcopenic obesity, defined as low muscle mass with high levels of adiposity, is associated with poor clinical outcomes (i.e., increased mortality). There is strong evidence that weight reduction has a dose-dependent relationship with the improvement and resolution of NAFLD, remarkably with a weight loss of >10%.[47-50] For every 1 kg of weight loss, a review of 43 studies reports a 0.83 U/L reduction in alanine aminotransferase (AST), a 0.56 U/L reduction in aspartate transaminase (AST), and a 0.77% reduction in steatosis assessed by radiology or histology.[51] Normal-weight NAFLD is defined as BMI ≤25 kg/m^2 and ≤23 kg/m^2 in non-Asian and Asian patients, respectively.

The American Gastroenterological Association (AGA) recommends a lower target weight-loss threshold for normal-weight NAFLD as it shows the following similar histologic benefits to NASH: fibrosis regression, steatosis improvement, decreased waist circumference, and decreased LDL levels. Non-alcoholic fatty liver disease resolution of 50% and 70% were observed with total body weight loss of 3%–5% and 7%–10%, respectively.[47]

Moreover, the concept of "hidden obesity" in sarcopenic patients is introduced as BMI ≤25 kg/m^2 with the addition of body fat percentage >25% in males and >30% in females. Hence, monitoring is emphasized in normal-weight NAFLD patients. Although presenting with less severe disease, they are susceptible to low skeletal muscle index attributed to decreased IGF-1 production; whereas in females, poor bone mass density, vitamin D3 deficiency, and a decrease in estrogen production.[52]

Diet

Patients with advanced liver diseases, including cirrhosis (i.e., a complication of NAFLD), are in an accelerated state of starvation and catabolism owed to increased gluconeogenesis, fat oxidation, ketogenesis, and impaired protein turnover.[53] To meet a patient's caloric and nutritional requirements, a consultation with a specialized nutritionist is preferred. The AGA 2019 recommends: 1) an individualized hypocaloric diet targeting 1200–1500 kcal/d or a reduction of 500–1000 kcal/d from baseline; 2) a minimum protein intake of 1.2–1.5 g/kg, favorably chicken, fish, eggs, nuts, lentils, and/or soy; 3) small frequent meals, <4–6 hours in between meals; 4) bedtime snack containing protein and ≥50 g of complex carbohydrates.[47] Similarly, the European Society for Clinical Nutrition and Metabolism (ESPEN) guideline recommends reducing periods of starvation by taking 3–5 meals/day and a late evening snack regardless of composition, to improve the total body protein

status and nitrogen balance, reverse anabolic resistance, and manage sarcopenia of cirrhosis.[53,54] A target protein intake of 1.2–1.5 g/kg/day is recommended for the upregulation of protein anabolism and improvement of total body protein in patients with cirrhosis and sarcopenia.[53]

In a Korean national survey with 4179 elderly participants with sarcopenia, the low protein intake group (<0.8 g/kg/day) had a significantly higher risk of sarcopenia (OR = 1.707). On the other hand, the high protein intake group (>1.2 g/kg/day) had lower prevalence of sarcopenia and sarcopenia-related factors such as insulin and total body fat.[46] However, a nationally representative data-based study demonstrated that participants with a higher sodium intake, as assessed by urinary sodium excretion, had significantly higher risks of both NAFLD (OR, 1.46, 95% CI, 1.27 to 1.66; p<0.001) and sarcopenia (OR, 1.49; 95% CI, 1.28 to 1.73; p<0.001). In addition, the study reported an average daily sodium consumption of 3.3 g in the general Korean population, which is higher than the WHO-recommended 2 g/day. This emphasizes the importance of optimal sodium intake for the general population, especially those at risk for both NAFLD and sarcopenia.[55]

Alcohol and smoking cessation

Although there is contradicting data on the effect of various amounts of alcohol consumption on NAFLD, the AGA 2019 guidelines recommend the best practice of complete alcohol restriction on adult patients with NAFLD.[47] A national prospective study reports a 22–40% reduction of incident cardiovascular disease with alcohol intake up to 49 g/day, but only in patients who were never smokers. Even low (0-9 g/day) alcohol intake produced elevated HRs for incident liver disease and cancer. Significantly elevated risks were shown for alcohol intakes of 10-19 g/day and >30 g/day.[56] The American Heart Association's Life's Simple 7 (LS7) metrics as surrogates of healthy living were investigated on subjects with NAFLD. Ideal glycemic control of hemoglobin A1c <5.7%, smoking status corresponding to a current nonsmoker and having smoked <100 cigarettes total, and BP level<120/80 mmHg offered significant protection against premature all-cause deaths. Moreover, smoking was the only ideal LS7 metric that had a higher prevalence among the group with sarcopenia compared to non-sarcopenia.[57]

Physical activity

The Expert Review of AGA 2019 recommends 1) 150–300 minutes of moderate-intensity or 75–150 minutes of vigorous-intensity aerobic exercise (i.e., walking, stationary biking) per week and 2) resistance training (i.e., weight lifting) as complementary, but not an alternative for adults with NAFLD.[47] In a systematic review by Hashida and colleagues, the median effective protocol for aerobic exercise was 4.8 metabolic equivalents (METs) for 40 min/session, 3 times/week for 12 weeks; whereas for resistance exercise, the median effective protocol was 3.5 METs for 45min/session, 3 times/week for 12 weeks.[58]

Specifically, activity management in sarcopenia-related NAFLD must be individualized, planned, and supervised.[59] For patients with poor cardiovascular fitness or any mobility-limiting comorbidity that may cause intolerance to aerobic training, resistance training is performed.[60] A systematic review of aerobic versus resistance training of equal duration, frequency, and period both showed improved hepatic steatosis, with no significant differences in BMI, ALT levels, and intrahepatic lipids. Total energy consumption (kcal/total period), percentage of maximum oxygen consumption, and metabolic equivalents were significantly lower in the resistance training group. On the other hand, energy consumption was lower in the aerobic exercise group (kcal/exercise).[58] Nonetheless, both types of training downregulate circulating inflammatory markers such as IL-6, CRP, and TNF-α.[61]

In a meta-analysis on the impact of endurance and combined training (i.e., endurance and resistance) on the sarcopenia criteria in NAFLD, both improved physical performance but not lean body mass. Considering most studies were endurance training protocols, this type of training may not positively impact muscle mass as compared to resistance training, which improves the power-producing capacity and sensitizes muscles to other anabolic stimuli needed in the setting of sarcopenia.

On the other hand, endurance exercise increases the oxidative capacity of skeletal muscle and supports faster protein turnover during resistance training.[61] Although the effect on muscle strength of the mentioned training cannot be determined due to a lack of studies, lower muscle strength, ideally evaluated through handgrip strength, is associated with a higher hepatic steatosis index.[59] To revert muscular mass loss, hypertrophic training is recommended: intensity of 40–80% of the individual in one maximum repetition, with loads >60% to increase maximal force and muscular mass.[62]

Meanwhile, an emphasis on lifestyle intervention in addition to MD was adopted for 6 months in an RCT, resulting in significant improvements in ALT levels and liver stiffness, whereas only liver stiffness improved with MD alone. The physical activity program included at least 30 min/d of moderate-vigorous activity (i.e., fast or very fast walking, slow or fast running, dancing, tennis), 10000 steps/d measured via pedometer given, and assessed via the validated Athens Physical Activity Questionnaire.[63] A similar study on the combination of aerobic physical activity and MD reduced the severity of liver steatosis and improved gut microbiota in a cohort of patients with NAFLD.[64]

Pharmacologic management

In patients who are unable to adhere to lifestyle interventions, pharmacological therapy is necessary. However, it should be emphasized that pharmacotherapy is not first-line and that there are no currently approved drugs for the

treatment of sarcopenia-associated NAFLD. Instead, we will discuss emerging treatment options for sarcopenia in chronic liver diseases.

β-Hydroxy-β-methylbutyrate

Beta-hydroxy-beta-methyl butyrate (HMB) has been evaluated to increase muscle mass and performance by promoting protein synthesis and counteracting muscle catabolism. In a single-blind RCT, 12 weeks of HMB supplementation (3 g/day) resulted in a statistically significant increase in muscle function assessed through a chair stand test and six-minute walk test, an increase in quadriceps muscle mass measured by ultrasound, and a decrease in liver function index compared to placebo (sorbitol powder).[65]

In malnourished cirrhotic patients, commercially available oral nutritional supplementations both provided by Abbott Laboratories (Madrid, Spain) were compared in a double-blind RCT: Ensure® Plus Advance (HMB group; 1.5 kcal/mL, 24.3% protein, 28.8% fat, and 1.5 g of calcium HMB per service) and 220 mL of Ensure® Plus High Protein (HP group; 1.25 kcal/mL, 25.3% protein, 23.8% fat). Improvement in liver function scores and BMI, with a reduction of LDL cholesterol, was observed in both groups. Although the HMB group had improved muscle strength and reduced minimal hepatic encephalopathy, larger trials are recommended before using HMB supplements.[66] In both RCTs, HMB supplementation was well tolerated by patients albeit with minimal gastrointestinal effects causing dropouts.

Branched-chain amino acid supplements

As a result of liver dysfunction and impaired ureagenesis, hyperammonemia occurs. The response of the body is to remove the excess extrahepatic ammonia occurring in the skeletal muscles through the synthesis of glutamine, in exchange for branched-chain amino acids (BCAAs). Moreover, there is an accelerated state of starvation causing increased gluconeogenesis and catabolism. Hence, there is decreased plasma BCAAs in patients with cirrhosis due to increased utilization as an energy source. Hyperammonemia-induced upregulation of myostatin, a negative regulator of muscle growth, is the key contributor to sarcopenia in patients with chronic liver disease.[53] There are ongoing clinical trials on the effect of BCAA supplementation on muscle mass, quality, and molecular markers of muscle regeneration in patients with chronic liver disease (NCT04246918) and liver cirrhosis (NCT03633279). The BCAA 10-gram-packet consists of 952 mg L-Isoleucine, 1904 mg L-Leucine, and 1144 mg L-Valine. In a single-center, prospective study of adult patients with cirrhosis, 24 weeks of oral BCAA supplement twice a day resulted in a significant increase in strength assessed via hand grip, but not in muscle mass.[67] Nonetheless, long-term BCAA supplementation (0.20–0.25 g/kg/day) has shown beneficial effects on protein metabolism (i.e., improved muscle mass) in patients with cirrhosis.[53]

Testosterone therapy

Low serum testosterone levels are seen in patients with decreased liver function, and liver cirrhosis, and even lower levels are seen in patients with sarcopenia and cirrhosis.[53] A clinical trial on the efficacy and safety of testosterone therapy in improving sarcopenia in men with cirrhosis (NCT03995251) is ongoing. In a 12-month, double-blinded, placebo-controlled trial in men with cirrhosis and total testosterone <12 nmol/L or free testosterone <230 pmol/L, serial administration of 1000 mg intramuscular testosterone undecanoate resulted in a significant increase in appendicular lean mass, a substantial increase in total lean mass, total bone mass, bone mineral density, hemoglobin, with reduced fat mass and HbA1c.[68]

KNOWLEDGE GAPS AND FUTURE DIRECTIONS

Non-pharmacologic management has been established as the first-line treatment for sarcopenia-associated NAFLD. Although uncertainties remain on the most effective diet and type, duration, and frequency of physical activity due to limited inter-trial comparisons, RCTs, and heterogeneity of the reviewed studies, optimal management is still individualized and supervised.[69] Socioeconomic factors that restrict patient's adherence to diet and exercise such as culture, younger age, lower income, and lower educational level should be investigated in community-based and large nationwide trials using consistent outcomes measures.[70,71] Key recommendations for future studies include local cuisine-based MD modifications, quality of life and need for palliative care referrals, complementary and alternative medicine, and pharmacologic options, especially for patients unable to adhere to lifestyle changes. This information is hoped to improve care, intervention sustainability, and acceptability for patients with sarcopenia-associated NAFLD.

Limitations

There are limitations to this narrative review that could affect how far its conclusions can be generalized. Aside from the aforementioned limitations (i.e., limited inter-trial comparisons, RCTs, and heterogeneity of the reviewed studies), there are no existing guidelines for sarcopenia and its interrelationship with non-alcoholic fatty liver disease. The relative variability and lack of uniformity (i.e., definition, diagnostic criteria, and monitoring) among the existing papers such as those seen in the European, International, and Asian Working groups may affect the generalizability of the study. Furthermore, possible confounders of the study are race and age distribution.

CONCLUSION

In this narrative review, we discussed the overlapping pathophysiologic mechanisms between NAFLD and sarcopenia, muscle-related factors influencing NAFLD progression, and liver dysfunction-related factors that promote sarcopenia. Nonpharmacologic management

remains the mainstay of treatment for NAFLD-associated sarcopenia: 1) screening and early diagnosis; 2) weight reduction; 3) diet; 4) alcohol and smoking cessation; and 5) resistance and hypertrophic training. For patients unable to perform the mentioned lifestyle interventions, clinical trials on pharmacologic options are ongoing to explore their utility.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement

FR: Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review and editing, Supervision, Project administration; **AA:** Formal analysis, Investigation, Data Curation, Writing – original draft preparation, Visualization, Project administration; **DC:** Formal analysis, Investigation, Data Curation, Writing – original draft preparation, Visualization; **NRB:** Formal analysis, Investigation, Data Curation, Writing – original draft preparation, Visualization; **SC:** Conceptualization, Validation, Writing – review and editing, Supervision, Project administration; **GPC:** Validation, Data Curation, Writing – review and editing, Visualization, Project administration; **MGY:** Conceptualization, Methodology, Validation, Resources, Writing – review and editing, Supervision; **EL:** Validation, Data Curation, Writing – review and editing, Visualization, Project administration; **KV:** Validation, Data Curation, Writing – review and editing, Visualization, Project administration.

Author Disclosure

The authors declared no conflict of interest.

Funding Source

None.

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REVIEW ARTICLE

Journal of the ASEAN Federation of Endocrine Societies

Efficacy and Safety of Bromocriptine-QR as an Adjunctive Therapy on Glycemic Control in Subjects with Uncontrolled Type 2 Diabetes Mellitus: A Systematic Review and Meta-analysis

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Abstract

Introduction. There has been an increasing awareness of the effects of combining bromocriptine-QR with other medications for diabetes mellitus type 2. This study aimed to assess the efficacy and safety of bromocriptine-QR as an adjunctive therapy for patients with uncontrolled type 2 diabetes mellitus.

Methodology. This systematic review is registered at the International Prospective Register of Systematic Reviews (CRD42022360326). Literature search was done via MEDLINE, NCBI, Google Scholar, Science Direct, Europe PMC and Cochrane Library databases. We included randomized controlled trials with participants 18 years old and above with uncontrolled type 2 diabetes mellitus. The primary outcome of interest is the efficacy and safety of bromocriptine-QR as an adjunctive therapy for glycemic control. Case reports, case series, reviews and animal studies were excluded. The risk of bias was reviewed using the Cochrane Risk of Bias tool. Meta-analysis was performed using Review Manager 5.4 and presented as a weighted mean difference and 95% confidence interval for changes from the baseline level.

Results. Nine studies were included in the systematic review with a total of 2709 participants. The baseline HbA1c in the bromocriptine-QR group was 7.42% and 7.51% in the control group. The bromocriptine-QR group was favoured, outperforming the control group in terms of reducing hemoglobin A1c(HbA1c), with a statistically significant difference (weighted mean difference -0.6%; 95% CI [-0.83,-0.36]; p<0.00001). The most common side effects were nausea (33.75% vs 6.92%), fatigue (13.11% vs 5.94%), and headache (11.17% vs 6.87%).

Conclusion. Administration of bromocriptine-QR at a dose range of 1.6 to 4.8 mg/day as an adjunctive therapy reduced HbA1c and FBG in patients with uncontrolled type 2 diabetes mellitus (T2DM). However, there were also statistically greater odds of the occurrence of adverse events such as nausea, vomiting, and headache compared to controls.

Key words: bromocriptine-QR, type 2 diabetes mellitus, HbA1c, side effects, glycaemic control, dopaminergic

INTRODUCTION

Diabetes mellitus can cause end-organ damage, leading to significant morbidity and mortality, such as cardiovascular disease (CVD), cerebrovascular events, renal failure, amputation and vision loss. Type 2 diabetes mellitus (T2DM) accounts for the vast majority (over 90%) of diabetes worldwide. The International Diabetes Federation 2021 stated that around 536.6 million people in the world live with diabetes, and is predicted to increase further by 2045. According to estimates, 19.5 million Indonesian people have diabetes, making it the country with the fifth greatest prevalence.[1]

Current recommendations from the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) include lifestyle modifications and oral hypoglycemic drugs (OHDs) as first-line therapy. Metformin, a biguanide, is the most recommended therapy for patients with T2DM. The selection of an add-on medication to metformin depends on patient preference and clinical characteristics, including comorbidities.[2]

The Food and Drug Administration (FDA) approved Bromocriptine-Quick Release (QR), a centrally-acting dopamine D2 receptor agonist, as the first medication to improve glycemic control by targeting dopamine activity in patients with T2DM. The dopaminergic pathway affects glucose and lipid metabolism, while patients with T2DM have been reported to have lower hypothalamic dopaminergic tone in the morning.[3] Dopamine agonists, such as Bromocriptine-QR, activate the dopaminergic

eISSN 2308-118x (Online)
Printed in the Philippines

Received: June 27, 2023. Accepted: September 3, 2023.
Published online first: February 21, 2024.
https://doi.org/10.15605/jafes.039.01.19

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pathway responsible for metabolic control, thus improving glucose and energy metabolism in patients with T2DM.[4]

Recognizing the effects of bromocriptine-QR has increased awareness of the possible role of combining this agent with other therapies for T2DM management. Hence, this study aimed to assess the efficacy and safety of bromocriptine-QR as an adjunctive therapy for glycemic control in people with uncontrolled T2DM.

METHODOLOGY

Eligibility criteria

This systematic review and meta-analysis are reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[5] The protocol of this review has been registered on the International Prospective Register of Systematic Reviews (PROSPERO) database with the registration number CRD42022360326.

We included randomized controlled trials (RCTs) with the population consisting of patients over 18 years old with uncontrolled type-2 diabetes mellitus (T2DM), defined as having HbA1c test results over 7.5% despite receiving standard regimens of oral hypoglycemic drugs, insulin, or both with stable doses. The bromocriptine group included patients who received bromocriptine-QR as an add-on to their respective anti-diabetic regimen. The comparators in the included studies (control group) were patients who were not given any additional treatment or those given a placebo. The primary outcome was to assess the efficacy and safety of bromocriptine-QR as an adjunctive therapy in patients with uncontrolled T2DM. The glycemic efficacy of bromocriptine-QR was evaluated based on the changes in baseline HbA1c and fasting plasma glucose (FPG) between the bromocriptine-QR group and the control group. All adverse effects reported by the included studies were also extracted to assess the safety and tolerability of bromocriptine-QR. We included studies with a duration of at least 12 weeks to assess the glycemic control of bromocriptine-QR adequately. The dose of bromocriptine-QR used in the included studies must be within 1.6 to 4.8 mg/day as approved by the Food and Drug Administration (FDA).[6]

Case reports, case series, reviews and animal studies were excluded. We also did citation and hand-searching to ensure that all available studies were included.

Search strategy and study selection

The literature search was conducted on September 5, 2022, and was finished on the same day. We systematically searched and obtained the papers from MEDLINE, NCBI, Google Scholar, Science Direct, Europe PMC and Cochrane Library. The search terms used included (“bromocriptine-QR") AND ("diabetes mellitus" OR "diabetes mellitus type 2" OR "diabetes mellitus type II") AND (“uncontrolled” OR "poorly controlled" OR "inadequately controlled"). The details of the Medical Subject Heading (MeSH) terms are listed in Table 1. All records were then inputted into Rayyan software, which can detect duplicates and allow all authors to collaborate in selecting relevant studies. Three authors (CLB, MM, SC) conducted the initial search and imported all studies from various academic databases to the Rayyan software. Another author (TY) would then cross-check all the initial searches. All authors independently screened all available studies. All conflicts met during the screening process were resolved by discussion among the group until a conclusion was reached. If any missing or further data were needed, corresponding authors were sent an email of inquiry once.

Data extraction and quality assessment

The data extraction process was done independently by three authors (CLB, MM and SC) and was checked by TY. The authors recorded study characteristics (author, year of publication, location, study design and study period),

Table 1. Medical subject heading (MeSH) terms used in each database

Database	Medical subject heading	Number of studies found
Pubmed	("bromocriptine"[MeSH Terms] OR "bromocriptine"[All Fields] OR "bromocriptin"[All Fields] OR "bromocriptine s"[All Fields] OR "bromocriptine-qr"[All Fields] OR ("bromocriptine"[MeSH Terms] OR "bromocriptine"[All Fields] OR "bromocriptin"[All Fields] OR "bromocriptine s"[All Fields] OR "cycloset"[All Fields])) AND ("diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR ("diabetes mellitus, type 2"[MeSH Terms] OR "type 2 diabetes mellitus"[All Fields] OR ("diabetes"[All Fields] AND "mellitus"[All Fields] AND "type"[All Fields] AND "ii"[All Fields]) OR "diabetes mellitus type ii"[All Fields]) OR ("diabetes mellitus, type 2"[MeSH Terms] OR "type 2 diabetes mellitus"[All Fields] OR "diabetes mellitus type 2"[All Fields])) AND ("uncontrollability"[All Fields] OR "uncontrollable"[All Fields] OR "uncontrollably"[All Fields] OR "uncontrolled"[All Fields] OR ("poorly"[All Fields] AND "controlled"[All Fields]) OR (("inadequate"[All Fields] OR "inadequately"[All Fields] OR "inadequates"[All Fields]) AND "controlled"[All Fields]))	12
NCBI	((bromocriptine) OR (bromocriptine-qr)) AND ((diabetes mellitus) OR (diabetes mellitus type 2) OR (diabetes mellitus type II))	1000
Google Scholar	(bromocriptine-qr) AND ("diabetes mellitus" OR "diabetes mellitus type 2" OR "diabetes mellitus type II") AND (uncontrolled OR "poorly controlled" OR "inadequately controlled")	181
Science Direct	(bromocriptine-qr OR dopamine agonist) AND (uncontrolled) AND (diabetes mellitus type II)	183
Medline	((bromocriptine) OR (bromocriptine-qr)) AND ((diabetes mellitus) OR (diabetes mellitus type 2) OR (diabetes mellitus type II))	10
Europe PMC	((bromocriptine OR bromocriptine-qr) AND ("diabetes mellitus") AND (uncontrolled or "poorly controlled")	127
Cochrane Library	((bromocriptine) OR (bromocriptine-qr)) AND ((diabetes mellitus) OR (diabetes mellitus type 2) OR (diabetes mellitus type II))	9

baseline HbA1c level, change of HbA1c level, treatment given to the intervention and control group, adverse effects, and baseline variables, including age, sex, body mass index (BMI) change, blood pressure (BP) change, creatinine, duration of T2DM and previous diabetic treatment regimens. Data presented as mean and standard error of the mean (SEM) were converted into mean and standard deviation using the Cochrane method.[7]

All authors independently assessed the quality of each included study using the Jadad Scale Assessment,[8] where a score of four and higher indicated higher-quality studies. We included moderate to high-quality studies. As for the risk of bias, we used the Cochrane Risk of Bias tool.[9] The overall certainty of evidence for each outcome was assessed using the Grading Recommendations Assessment, Development and Evaluation (GRADE) approach.[10] High certainty effects were characterized as the outcome effect, moderate certainty using "probably," low certainty using "may," and very low certainty using "uncertain." Any discrepancies were sorted internally until an agreement was attained.[11] Funnel plot was not generated as there was insufficient study.

Data synthesis

Review Manager 5.4 software was used to perform this meta-analysis. The primary outcome for glycemic control in this study is the change in HbA1c from baseline, and the secondary outcome is the fasting plasma glucose level. We calculated the weighted mean difference and 95% confidence intervals for changes from the baseline level in the bromocriptine-QR add-on group vs the control group. A fixed effect model was used when $I^2 \leq 50\%$ and $p > 0.1$, and a random effect model was used to merge the data when $I^2 > 50\%$ or $p < 0.1$. The degree of heterogeneity was assessed based on the I^2 statistic. A value of I^2 less than 25% was considered low heterogeneity, 26 to 50% was considered moderate heterogeneity, and greater than 50% was deemed high heterogeneity.[12,13] Subgroup analyses were done based on previous treatments to analyze the efficacy of adding bromocriptine to standard regimens.

RESULTS

Study selection and characteristics

The initial search yielded 1522 records, and 1357 were screened after removing duplicates. A total of 1190 studies were excluded after title and abstract screening, leaving 167 reports that were further assessed for eligibility. Animal studies, studies with incorrect study designs and outcomes, without full papers and those in non-English languages were excluded. Studies on patients with controlled diabetes and on the use of bromocriptine-QR as monotherapy were also excluded, leaving a total of seven studies. Additional hand-searching yielded two studies from Pijl et al.,[4] and Aminorroaya et al.[14] A total of nine studies were included in the systematic review and eight were eligible to be included in the meta-analysis (Figure 1).

Quality assessment

The studies included in this review were assessed using the Modified Jadad scale. All included studies were rated "high quality" based on the criteria (Table 2). In conclusion,

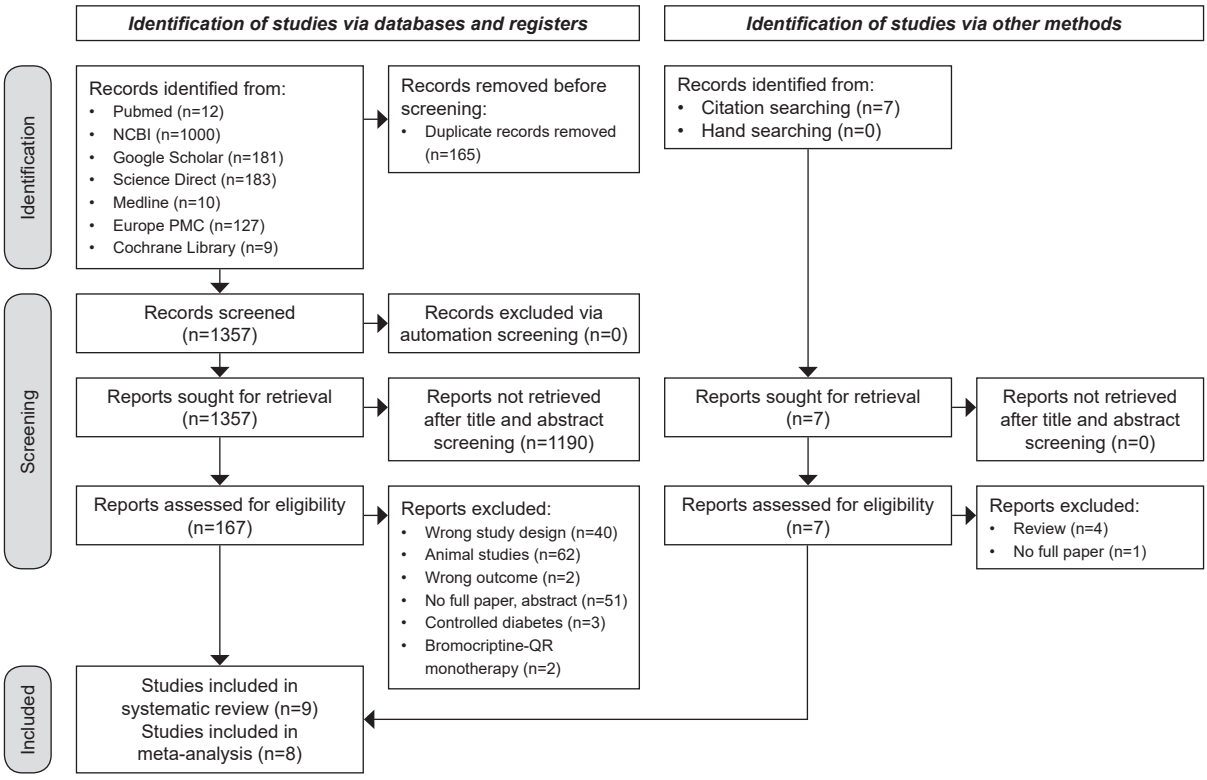

Figure 1. PRISMA flowchart for selection of included studies.

all studies were included in the review. The risk of bias is presented in Table 3.

Study characteristics and demographic data

The summary of each study and the summary of demographic data are presented in Tables 4 and 5, respectively. This review included 2709 patients, with 1800 patients in the bromocriptine-QR group and 909 in the control group. The mean (SD) age was 58.84 (8.87) years in the bromocriptine-QR group and 59.24 (8.98) years in the control group. Most participants in both groups were males, and the mean BMI was above 30. The mean (SD) duration of diabetes was 8.11 (6.46) years in the bromocriptine-QR group and 8.21 (6.2) years in the control group. Most patients in both groups used metformin monotherapy as their previous treatment (72.33% in the bromocriptine-QR group and 71.29% in the control group), and less than one percent of patients in both groups used sulfonylurea monotherapy as their previous regimen.

HbA1c outcome

The baseline mean HbA1c values in the bromocriptine-QR and control group were 7.42% and 7.51%, respectively. All studies reported a decline in HbA1c from baseline in the bromocriptine-QR group, while only three studies reported a decrease in HbA1c level in the control group. Meta-analysis favoured the bromocriptine-QR group in terms of HbA1c lowering when compared to the control group. The difference was deemed statistically significant (weight mean difference -0.6%; 95% CI [-0.83, -0.36]; p<0.00001). Moderate heterogeneity was found in the analysis (I^2 = 46%; random-effects modeling). Subgroup analysis was done to measure changes in HbA1C with metformin as previous anti-diabetic treatment (metformin + bromocriptine-QR vs. metformin/metformin + placebo). Analysis revealed no significant changes upon addition of bromocriptine-QR to metformin (weight mean difference –0.4%; 95% CI [–0.86, 0.060; p = 0.09, high certainty). Subgroup analysis using other previous anti-diabetic regimens was not performed due to a lack of data.

Fasting plasma glucose outcome

The baseline mean FPG values in the bromocriptine-QR and control groups were 152 mg/dL and 152.08 mg/dL, respectively. All studies reported a decreased FPG levels from the baseline in the bromocriptine-QR group. Meta-analysis showed a result favouring the bromocriptine-QR group in lowering FPG when compared to the control group, with a statistically significant difference (weight

Table 2. Quality appraisal of studies included in the meta-analysis using Modified Jadad scale assessment

Study	Was the research described as randomized?	Was the approach of randomization appropriate?	Was the research described as blinding?	Was the method of blinding appropriate?	Was there a description of with-drawals and dropouts?	Was there a presentation of the inclusion/ exclusion criteria?	Was the method used to assess adverse effects described?	Was the approach of statistical analysis described?	Total	Interpre-tation
Briones-Aranda, et al. (2018)[36]	1	1	0	0	0	1	1	1	5	High quality
Chamarthi, et al. (2017)[37]	1	1	1	1	0	1	1	1	7	High quality
Chamarthi, et al. (2016)[27]	1	1	1	1	0	0	1	1	6	High quality
Ghosh, et al. (2013)[38]	1	1	0	0	0	1	1	1	5	High quality
Vinik, et al. (2012)[15]	1	1	1	1	1	1	1	1	8	High quality
Florez, et al. (2011)	1	1	1	1	1	1	1	1	8	High quality
Ramteke, et al. (2011)[33]	1	1	1	1	0	1	1	1	7	High quality
Aminorroaya, et al. (2004)[4,14]	1	1	1	1	1	1	0	1	7	High quality
Pijl, et al. (2000)[4]	1	1	1	1	1	1	1	1	8	High quality

Table 3. Risk of bias in included studies based on Cochrane Risk of Bias Tool

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete complete data	Selective reporting	Other bias
Aminorroaya 2004	Low	High	High	High	High	High	Unclear
Briones-Aranda 2018	Low	High	Low	Low	High	High	Unclear
Chamarthi 2016	Low	High	High	High	High	High	Unclear
Chamarthi 2017	Low	High	High	High	High	High	Unclear
Florez 2011	High	Low	High	High	High	High	Unclear
Ghosh 2013	High	High	Low	Low	High	High	Unclear
Piji 2000	Low	High	High	High	High	High	Low
Ramteke 2011	High	High	High	High	High	High	Low
Vinik 2012	Low	Low	High	High	High	High	Unclear

Table 4. Summary of studies included

Author (year)	Study duration (week)	Study design (RCT)	Age, (years), mean ± SD		Baseline HbA1c level, mean ± SD (%)		Intervention (n)	Control (n)	HbA1c Change, % mean (95%CI)	
			B-QR	Control	B-QR	Control			B-QR	Control
Briones-Aranda, et al.(2018)[36]	12	Open-labeled	49.80 ± 20.36	50.50 ± 13.47	9.0 ± 1.13	9.3 ± 1.55	Metformin 850 mg + B-QR (gradually increased from 1.25 mg to 2.5 mg per day (10)	Metformin 850 mg (10)	-1.0 (0.08, 1.41)[c]	-0.6 (0.005, 1.14)[d]
Chamarthi, et al. (2017)[37]	12	Double-blind	59 ± 6.63	58 ± 12	8.31 ± 0.66	8.1 ± 0.8	Basal-bolus insulin + metformin + B-QR 1.6-4.8 mg/day (44)	Basal-bolus insulin + metformin + placebo (16)	-0.73 ± 1.06[a] (−1.05, −0.41)	+0.40 ± 1[a] (−0.14, 0.94)
Chamarthi, et al. (2016)[27]	12	Double-blind	59.5 ± 9.9	59.8 ± 9.7	[3]7.5	[3]7.5	Metformin + B-QR 1.6-4.8 mg/ day (1,208)	Metformin + Placebo (583)	-0.68[a]	-0.09[a]
Ghosh, et al. (2013)[38]	12	Open-labeled	50.92	48.1	7.90 ± 0.56	7.97 ± 0.56	Metformin 500 mg + B-QR 0.8 mg first weeks, others 1.6 mg (51)	Metformin 1000 mg/day (23)	-1.09[d]	-0.42[d]
Vinik, et al. (2012)[15]	24	Double-blind	57.9 ± 0.5	59.1 ± 0.7	8.28 ± 0.04	8.37 ± 0.06	One or two OAD (sulfonylurea/ thiazolidinedione/ alpha-glucosidase inhibitor/ meglitinide/ metformin) + B-QR 0.8-4.8 mg/day (341)	One or two OAD (sulfonylurea/ thiazolidinedione/ alpha-glucosidase inhibitor/ meglitinide/ metformin) (174)	-0.45[a] (-0.56, -0.34)	+0.06[a] (-0.1, 0.21)
Florez, et al. (2011)	52	Double-blind	56.4 ± 11	59.6 ± 10.9	8.2 ± 0.6	8.4 ± 0.7	Thiazolidinedione + B-QR 1.6-4.8 mg/day (78)	Thiazolidinedione + placebo (44)	-0.62 (-0.9, -0.34)[b]	+0.04 (-0.33, +0.42)[b]
Ramteke, et al. (2011)[33]	12	Double-blind	N/A	N/A	7.75 ± 0.50	7.73 ± 0.48	Metformin 1000 mg/day + B-QR 1.6 mg/day (33)	Metformin 1000 mg/day (32)	-0.74[c]	-0.63[d]
Aminorroaya, et al. (2004)[14]	12	Double-blind	50.6 ± 2.1	52.4 ± 2.0	9.9±0.3	10.2 ± 0.3	Glibenclamide or its combination with metformin + B-QR (gradually increased from 1.25 mg to 2.5 mg) (20)	Glibenclamide or its combination with metformin + placebo (20)	-0.4 (-1.7, 0.9)[d]	+1.1 (0.2, 1.9)[c]
Pijl, et al. (2000)[4]	16	Double-blind	56 ± 2	50 ± 3	8.7 ± 0.4	8.5 ± 0.5	Sulfonylurea + B-QR (15)	Sulfonylurea + placebo (7)	-0.6	+0.6

B-QR, bromocriptine-quick release; OAD, oral anti-diabetes drug; RCT, Randomized Controlled Trial; [a]$p \leq 0.001$; [b]$p < 0.01$; [c]$p < 0.05$; [d]$p \geq 0.05$

mean difference -1.08%; 95% CI [-1.62, -0.53]; p = 0.0001). High heterogeneity was found in the analysis (I^2 = 89%; random-effects modeling). In addition, subgroup analysis was performed to measure the change in FPG with metformin as previous anti-diabetic treatment (metformin + bromocriptine-QR vs. metformin/metformin + placebo), which yielded no significant changes (weight mean difference -1.31%; 95% CI [–2.73, 0.1]; p = 0.007, moderate certainty). The lack of data did not allow for all other planned subgroup analyses using other previous anti-diabetic regimens.

Certainty of evidence

The overall certainty of evidence for each outcome is described in Table 6.

Safety and tolerability

Adverse events that occurred more frequently in the bromocriptine-QR group than in the control group included nausea (33.75% vs. 6.92%), fatigue (13.11% vs. 5.94%), headache (11.7% vs. 6.87%), vomiting (9.06% vs. 3.08%), constipation (6.37% vs. 4.76%) and hypoglycemia (6.52% vs. 4.53%). Hypotension was rarely found in both groups (2.05% of the bromocriptine-QR group and 1.15% of the control group). Although not specified, the overall serious adverse events (SAEs) between the bromocriptine-QR group versus control were 5.79% and 7.72%, respectively. Serious adverse events in the cardiovascular system occurred less frequently in the bromocriptine-QR group than in the control group, as reported by Vinik et al.[15] (1.8% vs. 3.4%). Both studies also mentioned that SAEs in other organ systems occurred infrequently and had a similar percentage between the bromocriptine-QR and control groups. Meta-analysis showed that bromocriptine-QR has significantly greater odds of several adverse effects, such as nausea (OR 6.86; 95% 5.11 to 9.21, p < 0.0001), vomiting (OR 3.07; 95% 1.99 to 4.76, p <0.0001) and headache (OR 1.71; 95% 1.24 to 2.36, p = 0.001). However, side effects related to hypoglycemia (OR 1.45; 95% 0.99 tl 2.13, p = 0.06) and fatigue (OR 1.97; 95% 0.90 to 4.31, p = 0.09) are insignificant.

DISCUSSION

Bromocriptine mesylate is an ergot derivative that acts as a sympatholytic D2-dopamine receptor agonist, proposed as a novel centrally-acting antidiabetic agent.[4] This meta-

Table 5. Demographic data of the patients and reported adverse effects

Variable	Number of data available		n (%)	
	Bromocriptine-QR group	Control group	Bromocriptine-QR group	Control group
Age (years), mean ± SD	1716	854	58.84 ± 8.87	59.24 ± 8.98
Sex	1716	854		
Male, n (%)			977 (56.93)	510 (59.72)
Female, n (%)			739 (43.07)	344 (40.28)
BMI (kg/m²), mean ± SD	541	303	32.47 ± 3.28	31.77 ± 3.76
Systolic BP (mm/Hg), mean ± SD	1681	827	130.01 ± 12.58	129.23 ± 11.89
Diastolic BP (mm/Hg), mean ± SD	1681	827	77.15 ± 8.03	76.9 ± 8.06
Creatinine (mg/dL), mean ± SD	1262	609	1.01 ± 0.2	1 ± 0.2
Duration of diabetes (years), mean ± SD	1696	834	8.11 ± 6.46	8.21 ± 6.2
HbA1c (%), mean ± SD	1800	909	7.42 ± 1.06	7.51 ± 1.19
Fasting plasma glucose (mg/dL), mean ± SD	1790	899	152 ± 37.68	152.08 ± 38.38
Previous diabetes treatment	1800	909		
Metformin			1302 (72.33)	648 (71.29)
Thiazolidinedione			78 (4.33)	44 (4.84)
Sulfonylurea			15 (0.83)	7 (0.77)
Metformin and sulfonylurea			20 (1.11)	20 (2.2)
Two OADs, unspecified			341 (18.94)	174 (19.14)
Insulin basal-bolus and OAD			44 (2.44)	16 (1.76)
Serious adverse effects (SAEs)	673	337	39 (5.79)	26 (7.72)
Adverse effects				
Hypoglycemia	1671	817	109 (6.52)	37 (4.53)
Nausea	1600	780	540 (33.75)	54 (6.92)
Vomiting	1600	780	145 (9.06)	24 (3.08)
Hypotension	341	174	7 (2.05)	2 (1.15)
Headache	1549	757	173 (11.17)	52 (6.87)
Constipation	1549	757	88 (6.37)	36 (4.76)
Fatigue	1549	757	203 (13.11)	45 (5.94)

OAD, oral anti-diabetes drug

Table 6. GRADE Evidence Summary

Certainty assessment							No. of patients		Effect			
No. of studies	Study Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Bromocriptine	Control	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
HbA1C Changes												
8	randomized trials	not serious	not serious	not serious	not serious	none	952	326	–	SMD **0.6 SD lower** (0.83 lower to 0.36 lower)	⨁⨁⨁⨁ High	Important
FPG Change												
7	randomized trials	not serious	serious[a]	not serious	not serious	none	582	316	–	SMD **1.08 SD lower** (1.62 lower to 0.53 lower)	⨁⨁⨁◯ Moderate	Important

CI: confidence interval; SMD: standardized mean difference

Explanation
a. High I^2 statistics due to study differences

analysis showed a significant difference in HbA1c and FPG decline between the bromocriptine-QR and control group. These findings signify that oral bromocriptine-QR is superior to placebo for glycemic control in patients with uncontrolled T2DM. A study by Liang et al.,[16] found a similar conclusion, with bromocriptine-QR treatment lowering HbA1c more than the control group (weighted mean difference, -6.25 mmol/mol; 95% CI [-8.07, -4.97]).

The metabolic system of vertebrates during a food shortage inspired the use of bromocriptine for diabetes treatment. In such conditions, many develop obesity and insulin resistance. During the insulin-resistant state, dopamine levels are found to be low and later increase to normal after returning to the insulin-sensitive state.[17] Hence, reduced dopaminergic tone may be involved in the pathology of insulin resistance.[18] The development of an insulin-resistant state in animals closely resembles the changes in T2DM patients.[17] It is thought that people with type 2 diabetes experience a morning dip in dopaminergic tone, leading to increased sympathetic activity.[19] In addition, plasma prolactin is high in insulin-sensitive individuals during sleep. However, it was noted that in obese insulin-resistant individuals, daytime plasma prolactin levels were twice as high,[20] consistent with reduced dopaminergic tone.[21]

The possible mechanism driving the beneficial effects of bromocriptine-QR as adjunctive therapy in diabetes is the central modulation of dopaminergic and sympathetic tone.[21] Previous studies found that intracerebral injection of bromocriptine in insulin-resistant mice reduces noradrenergic and serotonergic levels, thereby improving

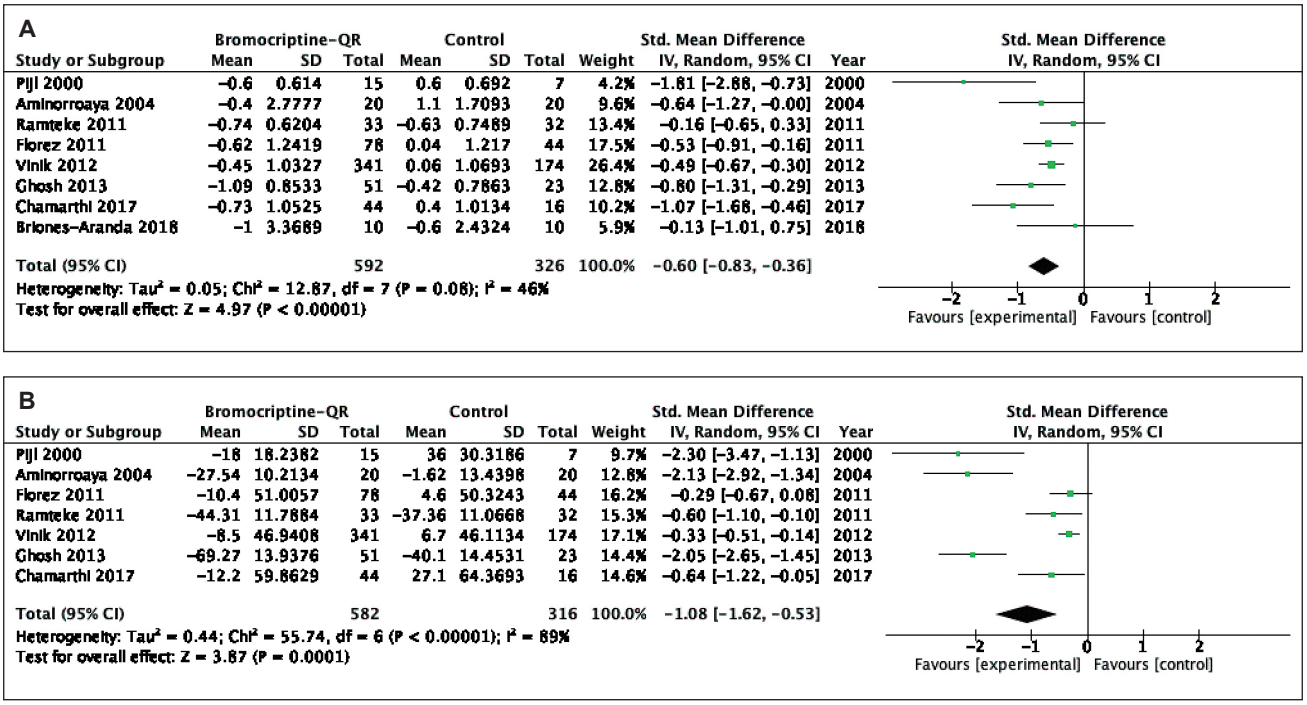

Figure 2. Forest-plot analysis for changes in HbA1c **(A)** and changes in fasting plasma glucose **(B)** in bromocriptine-QR and control group.

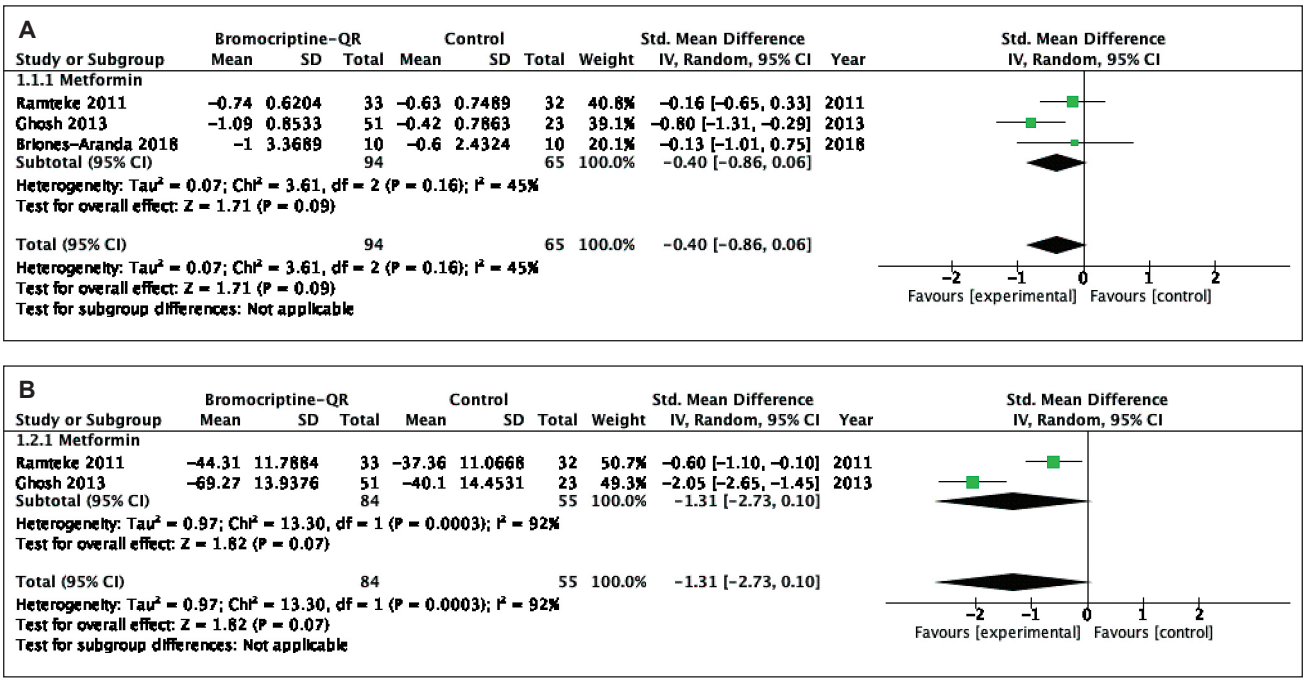

Figure 3. Subgroup analysis for changes glycemic index (HbA1c and fasting plasma glucose) based on metformin as previous anti-diabetic treatment (metformin + bromocriptine-QR vs metformin / metformin + placebo). **(A)** Changes in HbA1c value and **(B)** changes in fasting plasma glucose.

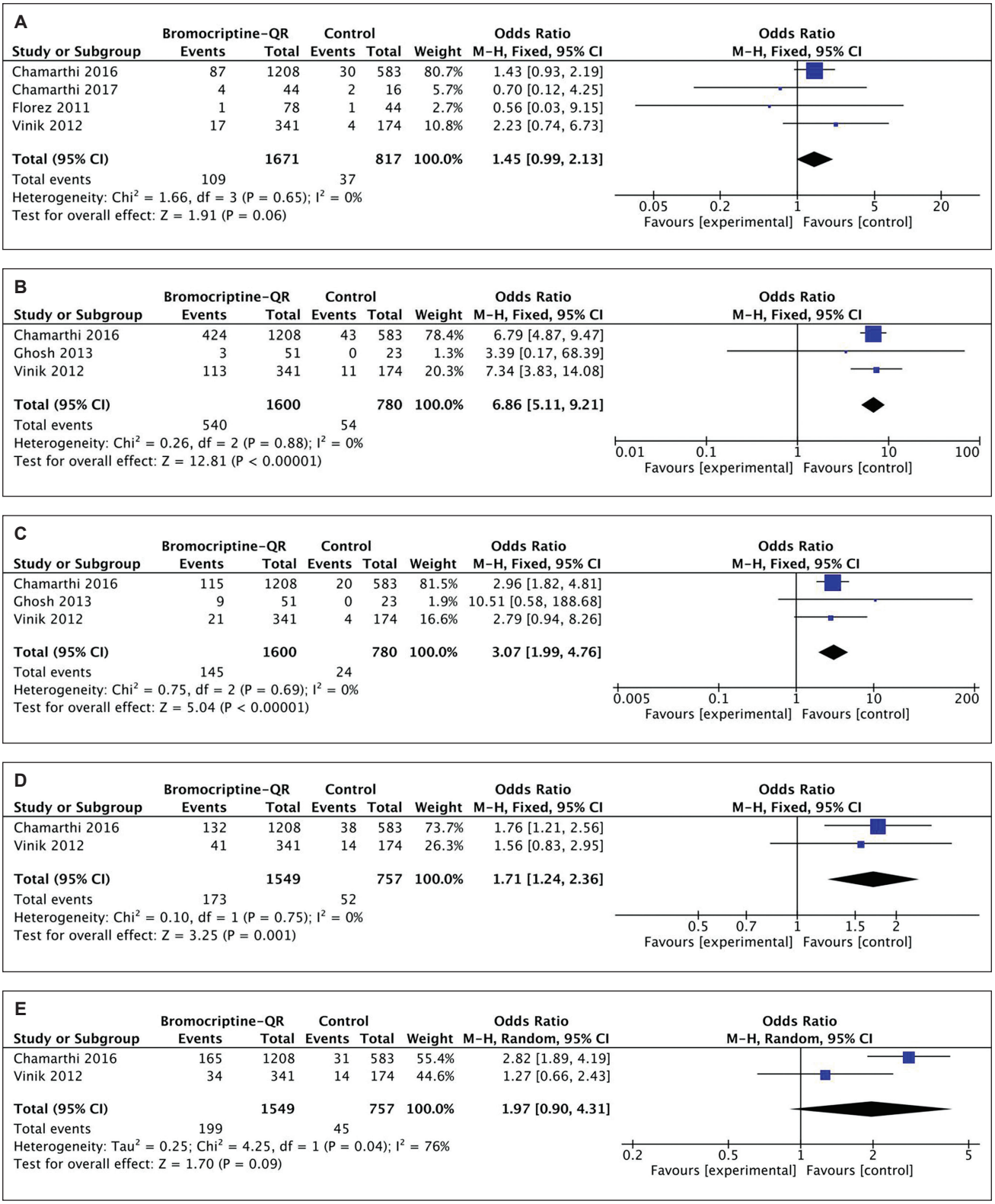

Figure 4. Forest-plot analysis for each adverse events of bromocriptine-QR group compared to control. **(A)** Hypoglycemia, **(B)** Nausea, **(C)** Vomiting, **(D)** Headache, **(E)** Fatigue.

insulin sensitivity and reducing plasma glucose.[22] The use of bromocriptine was assumed to increase dopaminergic activity in the morning and decrease sympathetic and serotonergic activity, leading to a decrease in insulin resistance and a decline in hepatic glucose output, hence improving glucose tolerance.[17,21]

Other mechanisms have been theorized regarding the use of bromocriptine-QR to improve glycemic control. It has been shown to inhibit glucose-stimulated insulin secretion by directly activating alpha2-adrenergic receptors in pancreatic beta cells. It also improves insulin sensitivity in hyperglycemic conditions by stimulating D2 receptors in beta cells. Reduced insulin resistance may also affect the gastrointestinal tract by suppressing hunger and enhancing satiation.[18]

It is well known that bromocriptine is used to treat Parkinson's disease and prolactinoma; however, bromocriptine used to treat T2DM is different.[17] Bromocriptine-QR was designed to provide a timed pulse of dopamine activity centrally by circulating brief daily intervals of bromocriptine at a particular time of the day. It differs from conventional bromocriptine, which is dosed several times daily and results in higher circulation of dopamine agonists, as used in Parkinson's disease. Bromocriptine-QR normalizes abnormal hypothalamic functions, decreases sympathetic tone and improves HPA axis circadian activity when administered at the appropriate time by restoring normal central dopaminergic activity in insulin-resistant subjects.[23]

This meta-analysis found a similar percentage of overall SAEs in both groups. The occurrence of SAEs in the cardiovascular system was less in the bromocriptine-QR group compared to the control group. Previous studies revealed that bromocriptine-QR has the potential for protective cardiovascular effects when given to T2DM patients.[23–27] Studies found that administration of bromocriptine-QR in T2DM subjects results in 40 to 55% relative risk reduction in CVD.[23,25–27] The promising cardiovascular benefit of bromocriptine-QR was also tested among children and adolescents with type 1 diabetes (T1DM) who are at risk of having vascular dysfunction and, therefore, present with a lifetime risk of CVD. A study found that bromocriptine-QR significantly reduces blood pressure and normalizes central and peripheral aortic stiffness over four weeks in youth with T1DM.[28]

The cardiovascular effects of bromocriptine-QR are not only explained by the reduction of fasting plasma glucose, HbA1c, plasma lipids, or blood pressure. The elevated sympathetic tone has been implicated in the pathophysiology of CVD. Bromocriptine-QR can reduce sympathetic activity by stimulating presynaptic dopamine receptors to inhibit norepinephrine release.[29,30] Moreover, evidence suggests that bromocriptine-QR has an anti-inflammatory effect by reducing endoplasmic reticulum (ER) stress genes, oxidative stress response genes and toll-like receptor (TLR) pro-inflammatory genes to substantially reduce the pro-oxidative/pro-inflammatory state in the development of CVD.[24] Bromocriptine-QR suppresses the vascular inflammations that lead to endothelial dysfunctions that are drivers of CVD.[31]

Other adverse events that occurred more frequently in the bromocriptine-QR group than in the control group include gastrointestinal effects (nausea, vomiting and constipation), fatigue, headache and hypoglycemia. These reported side effects were classified as mild to moderate. The relatively lower doses of bromocriptine-QR used in the treatment of T2DM compared to the usual doses in hyperprolactinemia or Parkinson's disease play a part in reducing the risk of adverse effects. The common adverse effects can be minimized by gradual weekly titration of the bromocriptine-QR dosage.[32] Although hypoglycemia was found more frequently in the bromocriptine-QR group than in the control group, the episodes were mild, transient and resolved spontaneously after food intake.[15,33] The mild adverse effects may limit bromocriptine-QR use in some patients; however, studies mentioned that bromocriptine-QR has several favorable clinical properties, such as a low degree of weight loss or weight gain, and it is not strongly associated with hypoglycemia.[4,34,35] The low rate of hypoglycemia is explained by how bromocriptine-QR does not stimulate insulin secretion but improves insulin sensitivity.[21]

The results of this meta-analysis should be seen in the light of a few limitations. Some studies included had limited data on the safety and tolerability of using bromocriptine-QR in T2DM patients. The included studies also appear to be short-term observations; hence, bromocriptine-QR needs further evaluation for long-term efficacy, safety and tolerability. Based on the I^2 value, this meta-analysis has moderate to high heterogeneity. Small study effects, clinical variations such as the time of HbA1c or plasma glucose measurement and sampling methodology may be the source of unexplained heterogeneity. The wide range of HbA1c values and various types of adjunctive therapy in combination with bromocriptine-QR used among the included studies may have also introduced heterogeneity in this study.

Despite these limitations, our study also has its merits. We included nine studies with over 1700 uncontrolled T2DM patients receiving bromocriptine-QR as an adjunctive treatment. This is considered a large analysis comparing the addition of bromocriptine-QR to the standard of care. In addition, we also included clinical trials to give more complete data about the efficacy and safety of bromocriptine-QR as an anti-diabetes drug.

CONCLUSION

This meta-analysis found that administration of bromocriptine-QR at a dose range of 1.6 to 4.8 mg/day as an adjunctive therapy has favourable outcomes in T2DM

as it significantly reduces HbA1c and fasting plasma glucose compared to placebo. Although bromocriptine-QR led to numerically greater adverse events such as nausea, vomiting and headache, they are generally mild. It is relatively safe and tolerable among T2DM patients under short-term surveillance. Bromocriptine-QR may reduce the risk of adverse cardiovascular events in T2DM. From these findings, it can be an alternative candidate to further expand well-established treatment options in T2DM patients, especially those on injectable medications.

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

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

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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Journal of the ASEAN Federation of Endocrine Societies

REVIEW ARTICLE

Effects of Combination of Curcumin and Piperine Supplementation on Glycemic Profile in Patients with Prediabetes and Type 2 Diabetes Mellitus: A Systematic Review and Meta-analysis

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Abstract

Objective. This study aimed to evaluate the effects of the combination of curcumin and piperine supplementation on Fasting Plasma Glucose (FPG), Homeostatic Model of Insulin Resistance (HOMA-IR), and Body Mass Index (BMI) in patients with prediabetes and type 2 Diabetes Mellitus (T2DM). This review was done to identify potential herbal remedies that may help improve glycemic parameters, leading to better health outcomes in combination with current antidiabetic treatment.

Methodology. This systematic review was based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). It was conducted in 2023 with sources and databases from MEDLINE, EBSCO-Host, ScienceDirect and ProQuest. This paper included randomized-controlled trials exploring the effects of the combination of curcumin and piperine on patients with prediabetes and T2DM. Systematic reviews, observational studies, case reports, case series, conference abstracts, book sections, commentaries/editorials, non-human studies and articles with unavailable full-text and written in non-English language, were excluded. The key terms for the literature search were "curcumin," "piperine," "prediabetes" and "Type 2 Diabetes Mellitus." We use Cochrane Risk of Bias (RoB) 2 for quality assessment of the included studies and Review Manager (RevMan) 5.4 to do the meta-analysis.

Results. A total of three studies were included in this systematic review. Two studies from Neta et al., and Cicero et al., showed no significant difference in HOMA-IR, BMI and FPG levels between the curcumin, piperine and placebo groups. One study from Panahi et al. demonstrated a significant difference in BMI levels between the curcumin and piperine and placebo groups (p <0.01). The meta-analysis showed that FPG levels, HOMA-IR and BMI improved among patients with diabetes given in curcumin and piperine with reported mean differences (MD) of = -7.61, 95% CI [-15.26, 0.03], p = 0.05, MD = -0.36, 95% CI [-0.77 to 0.05], p = 0.09, and MD = -0.41, 95% CI [-0.85 to 0.03], p = 0.07, respectively).

Conclusions. The supplementation of curcumin and piperine showed a numerical reduction in FPG, HOMA-IR and BMI, but were not statistically significant. Further research is needed as there is a paucity of studies included in the review.

Key words: glycemic profile, curcumin, piperine, prediabetes, type 2 diabetes mellitus

INTRODUCTION

Type 2 Diabetes mellitus (T2DM) is a chronic condition characterized by hyperglycemia. The term prediabetes is used to describe a state between normality and diabetes.[1] The latest survey conducted in 2021 by the International Diabetes Federation (IDF) showed that 537 million adults worldwide and about 90 million in Southeast (SE) Asia are living with diabetes. These numbers affected the overall health and well-being of patients. Diabetes causes serious complications, resulting in a high mortality rate of 6.7 million deaths worldwide (around 747,000 in SE Asia) in 2021.[2] Moreover, it contaminates the quality of life (QoL) with a disability-adjusted life year (DALY) sum of 66.3 million in 2019.[3] Total health expenditures reached up to 996 billion USD.[2]

Chronic hyperglycemia contributes to the production of Advanced Glycation End products (AGEs) and Reactive Oxidative Species (ROS), which increase the risk of microvascular and macrovascular complications. Various medications to combat hyperglycemia and its

eISSN 2308-118x (Online)
Printed in the Philippines

Received: June 28, 2023. Accepted: August 8, 2023.
Published online first: February 17, 2024.
https://doi.org/10.15605/jafes.039.01.18

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complications have been developed. These medications include insulin analogs and oral medications that improve insulin sensitivity, increase insulin production, inhibit glucose absorption and more.[4] However, these benefits have disadvantages, such as high costs and some adverse effects.[5] Alternatives, such as herbal remedies, were created to minimize these drawbacks without compromising the benefits of modern drugs. Furthermore, these remedies provide anti-inflammatory and antioxidant effects that could potentially prevent complications.[6]

Curcumin, an active compound of turmeric, is among the most well-studied herbal remedies. It has antidiabetic properties as it can reduce hepatic glucose production by activating AMP kinase and inhibit both glucose-6-phosphatase and phosphoenolpyruvate carboxykinase activity; therefore it may potentially prevent T2DM complications by reducing oxidative stress.[6] It may also play a role in improving fasting plasma glucose (FPG), HbA1c, body mass index (BMI), lipid profile and insulin sensitivity.[7,8]

Another herbal remedy of interest as a diabetic medication is piperine, a chemical compound found in black pepper. Its therapeutic benefits in diabetes are similar to curcumin. Giving piperine in combination with curcumin was found to have a synergistic effect. Piperine increases curcumin bioavailability, either through increased absorption or reduced metabolism.[10] Individually, these herbal remedies were extensively studied, however, they are yet to be reviewed in combination. Therefore, this systematic review and meta-analysis aimed to evaluate the effects of combined curcumin and piperine supplementation on the glycemic profile of patients with prediabetes and T2DM.

METHODOLOGY

This systematic review was designed and conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) 2020 statement guideline.[11]

Eligibility criteria

Types of studies

This systematic review included all published and unpublished randomized-controlled trials that investigated the effects of the combination of curcumin and piperine supplementation on patients with prediabetes and T2DM. Conversely, reviews, cross-sectional studies, cohort studies, case reports, case series, conference abstracts, book sections, commentaries/editorials and non-human studies were excluded. Articles not in English and without available full-text manuscripts were also excluded.

Participants

All patients aged ≥18 with prediabetes and T2DM were included in this study. Diagnosis of prediabetes and type 2 diabetes mellitus was based on the American Diabetes Association criteria.[12] Patients were diagnosed with T2DM if glycated hemoglobin (HbA1c) was ≥6.5% or fasting plasma glucose (FPG) was ≥126 mg/dL. Prediabetes was diagnosed at an HbA1c level of 5.7 to 6.4% or FPG of 100 to 125 mg/dL. There were no limitations for sex and race. Patients with current glucocorticoid use, cardiovascular disease, kidney failure, inflammatory diseases, acute infections and who were pregnant or breastfeeding were excluded from the study.

Variable of interest

Our study aimed to evaluate the effect of combined curcumin and piperine supplementation on glycemic profile in patients with prediabetes and T2DM.

Outcome of interest

Outcomes of interest in this study were the changes in fasting plasma glucose (FPG), homeostatic model assessment for insulin resistance (HOMA-IR), and body mass index (BMI) before and after intervention with combined curcumin/piperine. Other glycemic and metabolic parameters, such as hormonal and lipid parameters, inflammatory biomarkers and oxidative molecules, were considered additional outcomes.

Search strategy and study selection

We used MEDLINE, EBSCO-Host, Science Direct and ProQuest electronic databases to search for eligible studies as of 2023. EBSCO-Host and ProQuest databases were also screened for grey literature to identify unpublished studies with suitable PICO criteria. Five independent authors identified eligible studies by using the following keywords: *(Type 2 Diabetes Mellitus or Diabetes Mellitus, Noninsulin-Dependent or NIDDM or Maturity-Onset Diabetes Mellitus or Diabetes Mellitus, Type 2 or Prediabetes or Prediabetic State OR Prediabetic States) and (curcumin or curcuma or curcuminoid) and (Piperidines OR Piperine).*

All obtained studies were exported into the Mendeley reference manager software. The authors independently reviewed and screened these studies for duplicates. Studies with titles and abstracts unbefitting for this paper's objectives were excluded. Full texts of the selected studies were thoroughly assessed using the eligibility criteria described above, and those eligible were subsequently included in this review. The review team resolved any disagreements.

Data collection process

All studies were analyzed and the following data were extracted: first author, country of origin, study design, sample sizes, age, sex, prediabetes and diabetes criteria, curcumin and piperine administration protocol, adjusted confounding factors/population matching and the outcome of interest.

Summary measures

HOMA-IR, FPG and BMI for patients in the curcumin plus piperine group and placebo group were measured and reported as numerical (continuous) data. The data were presented in mean ± standard deviation for normally distributed data or median (interquartile range) for non-normally distributed data. The p-value and confidence interval were also included for each item to determine significance.

Assessment of risk of bias/ Quality assessment

Each study was assessed using the Cochrane Risk of Bias Tool 2.0 (RoB 2) for randomized controlled trials.[13] The tool consists of seven main domains: (a) Random Sequence Generation; (b) Allocation Concealment; (c) Blinding of Participants and Personnel; (d) Blinding of Outcome Assessment; (e) Incomplete Outcome Data; (f) Selective Reporting; and (g) Other Source of Bias. From each domain, the risk of bias was considered as low, high, and moderate bias. Each trial's overall quality was divided into three groups based on the degree of bias present: (1) low risk of bias (low risk of bias across all domains); (2) high risk of bias (high risk of bias across multiple domains); and (3) some concerns some concerns across at least one domain). Two reviewers evaluated each article separately, and disagreements were discussed among the whole review team until agreement was obtained.

Synthesis of results and statistical analysis

For all continuous outcomes, we calculated the standardized mean differences (SMDs) and 95% Confidence Intervals (CIs) based on the mean changes from baseline to the end of the study from each group. Statistical analyses were done for the between-group comparison. Missing data, such as Standard Deviation (SD), were calculated from the Standard Error (SE). SE of a Mean Difference (MD) was calculated from a p-value by finding the associated t-value. Having calculated the SE of the MD, the SD can be calculated from the SE. Because some studies reported primary outcomes using different evaluation or calculation methods, the meta-analyses were conducted with a random effects model. This model presupposes that the treatment impact will be distributed over certain populations, giving each study an equal weighting. The combined effect measures of the direct comparisons from individual interventions were compared by using the inverse variance method.

Heterogeneity across trials was assessed using the I^2 statistic. An I^2 value of less than 25% is considered low heterogeneity, between 25% and 50% indicates moderate to substantial heterogeneity, and more than 50% is considered high heterogeneity.[14] When heterogeneity was present, possible causes were investigated via sensitivity analyses. A p-value <0.05 was considered significant. A continuous outcome was analyzed as a weighted mean difference. Differences across studies were calculated based on population sample sizes. In addition, publication bias was evaluated visually using a funnel plot, in which the effect of each trial was plotted by the inverse of its SE. All analyses were conducted using RevMan software version 5.4.[15]

Quality assessment in the cumulative evidence

Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) was described to determine the confidence in cumulative evidence. The decision was made by considering several aspects of the studies, such as study limitations, consistency, directness, precision and reporting bias. The evidence results were graded as very low, low, moderate and high.

RESULT

PRISMA

A flow chart of the research selection process and its results are summarized in Figure 1. The search strategy yielded 456 potentially relevant studies. According to the selection criteria, ten studies were identified for further full-text assessment, of which five articles were review articles (non-primary articles), one was an in-vitro study, and one was an in-vivo study. No unpublished studies were included, minimizing publication bias qualitatively. Finally, three studies were included in the systematic review and three studies eligible for data extraction were included in the meta-analysis. All studies were published between 2003 and 2023.

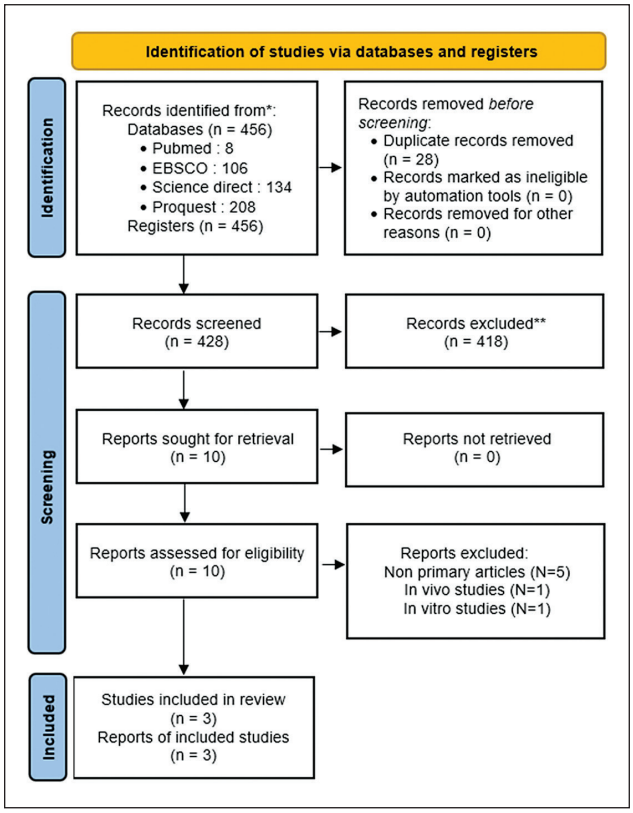

Figure 1. PRISMA 2020 Flow Diagram of Included Studies.

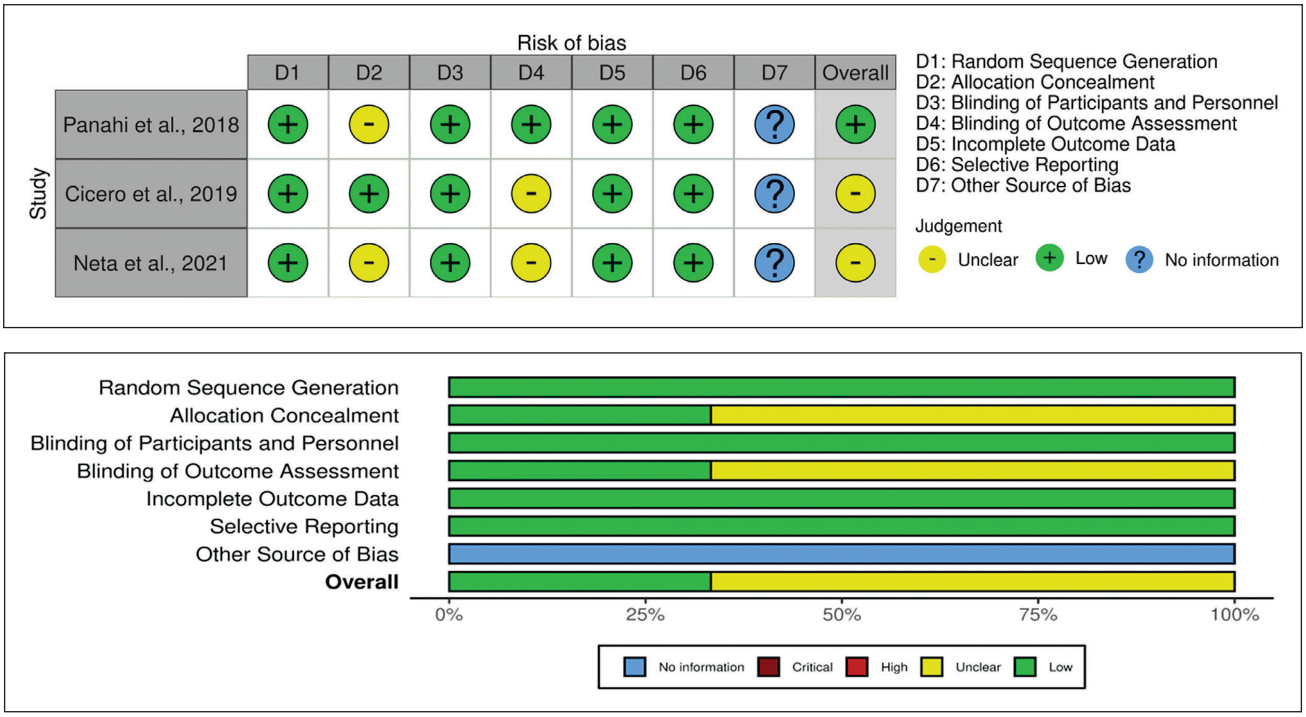

Figure 2. Results of Study Quality Assessment.

Quality assessment

Quality assessment for obtained studies was performed using the Cochrane Risk of Bias Tool 2.0 (RoB 2) for randomized-controlled trials (Figure 2). We obtained one low-bias study[16] and two unclear bias studies.[17,18] Across all parameters of bias, two studies[16,18] reported an unclear bias of the allocation concealment, and two studies[17,18] reported an unclear bias in the blinding of outcome assessment.

Characteristics of the included studies

Three randomized-controlled studies met the inclusion criteria. These studies included 123 patients receiving a combination of curcumin/piperine and 118 patients in the placebo groups. The characteristics of the included studies, including the number of participants (N), age (years), prediabetes and diabetes diagnosis, curcumin and piperine administration protocol, adjusted confounding factors/population matching, and the outcome of interest were extracted from each study and reported in Table 1. The mean ages of the intervention groups compared with the placebo groups ranged from 43 ± 8 to 63.1 ± 11.1 years and 41 ± 7 to 61.9 ± 11.0 years, respectively. One study[16] did population matching of baseline characteristics at the beginning of the study, and the other two studies[17,18] matched the population in terms of diet and physical activity. Out of the three studies, one study[18] was conducted in Brazil and the other two studies[16,17] were conducted in Iran. The inclusion criteria, dosages and frequency of taking curcumin varied across studies. The participants of each study were adults, either male or female, diagnosed with T2DM, except in Cicero et al.,[17] who used subjects with no T2DM, but with prediabetes.

Final results

All three studies included in this systematic review showed insignificant differences in Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) between the intervention and placebo groups, as shown in Table 2**.** Insignificant differences were also shown in all studies for the Fasting Plasma Glucose (FPG) levels, as shown in Table 3**.** A randomized-controlled study by Panahi et al.,[16] showed a significant difference in body mass index (BMI) between the two groups, while the rest showed otherwise (Table 4). There were no reports of attrition with regard to treatment during the follow-up period. Furthermore, all included studies reported no adverse effects during the treatment duration**.**

Meta-analysis results

The results of quantitative synthesis for each outcome (FPG, HOMA-IR and BMI) are reported in continuous data as mean differences (MD), as shown in Figures 1, 2 and 3, respectively. In one study, fasting plasma glucose is proven to be lower in curcumin-piperine groups compared to placebo groups (p=0.05), with a mean difference of -7.61, but analyzed together, the results are not significant due to the overall estimates of its forest plot intersecting with the line of no effect (95% CI [-15.26, 0.03]). The most significant differences in FPG between the two groups before and after the intervention are observed in Neta et al., with a mean difference of -19.10 (95% CI [-30.84, -7.36])[18] and the smallest is shown in Cicero et al., with the mean difference of -2.00 (95% CI [-9.06, 5.06]).[17] HOMA-IR and BMI are found to be numerically lower in curcumin-piperine groups but not statistically significant, with both overall

Table 1. Study characteristics

No.	Author, publication year, country	Types of Study Curcumin Piperine	Population				Curcumin piperine and placebo administration protocol	Prediabetes and diabetes criteria	Adjusted confounding factors/population matching	Outcome of interest
			Total (N)		Age (years)					
			Curcumin piperine	Placebo	Curcumin piperine	Placebo				
1	Panahi et al., 2018, Iran[16]	RCT	Male: 25 Female: 25	Male: 26 Female: 24	43 ± 8	41 ± 7	- Intervention Groups: Curcuminoids (Curcumin C3 Complex®, Sami Labs LTD, Bangalore, India; 500mg/day). The curcuminoids preparation (C3 Complex®) contained curcumin, demethoxycurcumin and bisdemethoxycurcumin in a patented ratio. Each curcuminoids capsule also contained 5 mg piperine (Bioperine®; Sami Labs LTD, Bangalore, India). - Control Groups: Placebo (not further explained). - Duration of treatment: 3 months	T2D based on fasting plasma glucose ≥126 mg/dL, glycated hemoglobinA1c (HbA1c) ≥6.5 %, or the use of standard anti-diabetic treatments.	Patients' characteristics were considered similar through randomization, but there is no further information regarding the adjusted confounding factors.	Fasting serum concentrations of insulin, glucose, HbA1c, C-peptide, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and high-sensitivity C-reactive protein (hs-CRP), homeostatic model assessments (HOMA) of insulin resistance (HOMA-IR) and beta-cell function (HOMA-β)
2	Cicero et al., 2019, Iran.[17]	RCT	Male: 18 Female: 22	Male: 19 Female: 21	54 ± 3	53 ± 5	- Intervention Groups: Tablets containing 800 mg phytosomal curcumin (Curserin®: 200 mg curcumin, 120 mg phosphatidylserine, 480 mg phosphatidylcholine, associated to 8 mg piperine from *Piper nigrum* L. dry extract) taken 2 tablets per day after dinner. - Control Groups: Placebo (not further explained). - Duration of treatment: 3 months	FPG levels between 100 and 125 mg/dL	During the study, subjects were encouraged to follow basic guidelines for a Mediterranean diet, refrain from consuming too much dairy and food derived from red meat, and keep generally stable eating patterns. Additionally, people were urged to boost their physical activity by cycling or walking briskly for 20-30 mins, 3-5x each week.	Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), LDL-Cholesterol (LDL-C), fasting plasma glucose (FPG), glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and gamma-glutamyl transferase (gamma-GT)
3	Neta et al., 2021, Brazil.[18]	RCT	Male: 10 Female:23	Male 4 Female: 24	63.1 ± 11.1	61.9 ± 11.0	- Intervention Groups: 500 mg of *Curcuma longa* L. to be ingested once a day on an empty stomach. 5 mg piperine was added to every capsule - Control Groups: Placebo (500 mg of carboxymethyl cellulose once a day on an empty stomach for 120 days - Duration of treatments: 3 months	Not stated	Participants were advised not to make any special changes in their diet and physical activity, as well as to report any changes in medications during the intervention period.	(LDL-C), fasting plasma glucose (FPG), glutamic-oxaloacetic

estimates crossing the line of no effect (p=0.09 and p=0.07, mean differences of -0.36, 95% CI [-0.77, 0.05] and -0.41, 95% CI [-0.85, 0.03], respectively).

DISCUSSION

Population characteristics, such as ethnicity, play a significant role in treatment response. It is found that ethnicity influences insulin sensitivity, thereby improving glycemic control.[19] Moreover, it also greatly affects the pharmacokinetic properties of therapeutic substances.[20] A study conducted by Viana et al., in Brazil,[21] which is similar to Neta et al.,[18] found that DM patients have poor glycemic control despite treatment.[21] Meanwhile, Panahi et al.,[16] and Cicero et al.,[17] discovered that the treatment has a crucial effect on a person's glycemic control.[21] There is also a difference in the spectrum of hyperglycemia (prediabetes and DM) among the three studies. Diabetes mellitus is the progression of prediabetes, which is characterized by worsening glycemic control and insulin resistance necessitating treatment intensification.[22] Furthermore, differences were noted regarding the use of anti-diabetic drugs between the studies. Although the efficacy of these drugs is clearly stated,[4] it can be disregarded since each study stated the baseline parameters and no changes were made in anti-diabetic drugs used. All these dissimilarities could potentially alter the overall results of the studies.

Baseline parameters are primarily similar across the three studies. The average BMIs are classified as overweight-

Table 2. Changes in Homeostatic Model Assessment For Insulin Resistance (HOMA-IR) before and after intervention

No	Author, Year Curcumin + Piperine	HOMA-IR			
		Curcumin + Piperine	Placebo	Mean difference (95% CI)	*p**
1	Panahi et al., 2018[16]	− 0.2 ± 0.4	− 0.1 ± 0.3	-0.10 (-0.24, 0.04)	0.511
2	Cicero et al., 2019[17]	− 1.1 ± 0.6	-0.5 ± 0.2	-0.60 (-0.80, -0.40)	0.013
3	Neta et al., 2021[18]	-0.58 ± 1.48	-0.16 ± 1.25	-0.42 (-1.11, 0.27)	0.458

Table 3. Changes in Fasting Plasma Glucose (FPG) before and after intervention

No	Author, Year Curcumin + Piperine	FPG			
		Curcumin + Piperine	Placebo	Mean difference (95% CI)	*p**
1	Panahi et al., 2018[15]	− 9 ± 16	− 3 ± 11	-6.00 (11.38, -0.62)	0.048
2	Cicero et al., 2019[17]	− 7 ± 3	-5 ± 22.56	-2.00 (-9.06, 5.06)	0.317
3	Neta et al., 2021[16]	-6.6 ± 27.7	12.5 ± 18.8	-19.10 (-30.84, -7.36)	0.630

Table 4. Changes in Body Mass Index (BMI) before and after intervention

No	Author, Year Curcumin + Piperine	BMI			
		Curcumin + Piperine	Placebo	Mean Difference (95% CI)	*p*-value**
1	Panahi, et al. 2018[16]	− 0.5 ± 0.5	0.2 ± 0.7	-0.70 (-0.94, -0.46)	<0.001
2	Cicero et al., 2019[15]	− 0.8 ± 0.2	-0.5 ± 2.7756	-0.30 (-1.16, 0.56)	0.286
3	Neta et al., 2021[17]	-0.13 ± 0.3	0.02 ± 0.76	-0.15 (-0.45, 0.15)	0.387

*significant if p-value ≤0.05

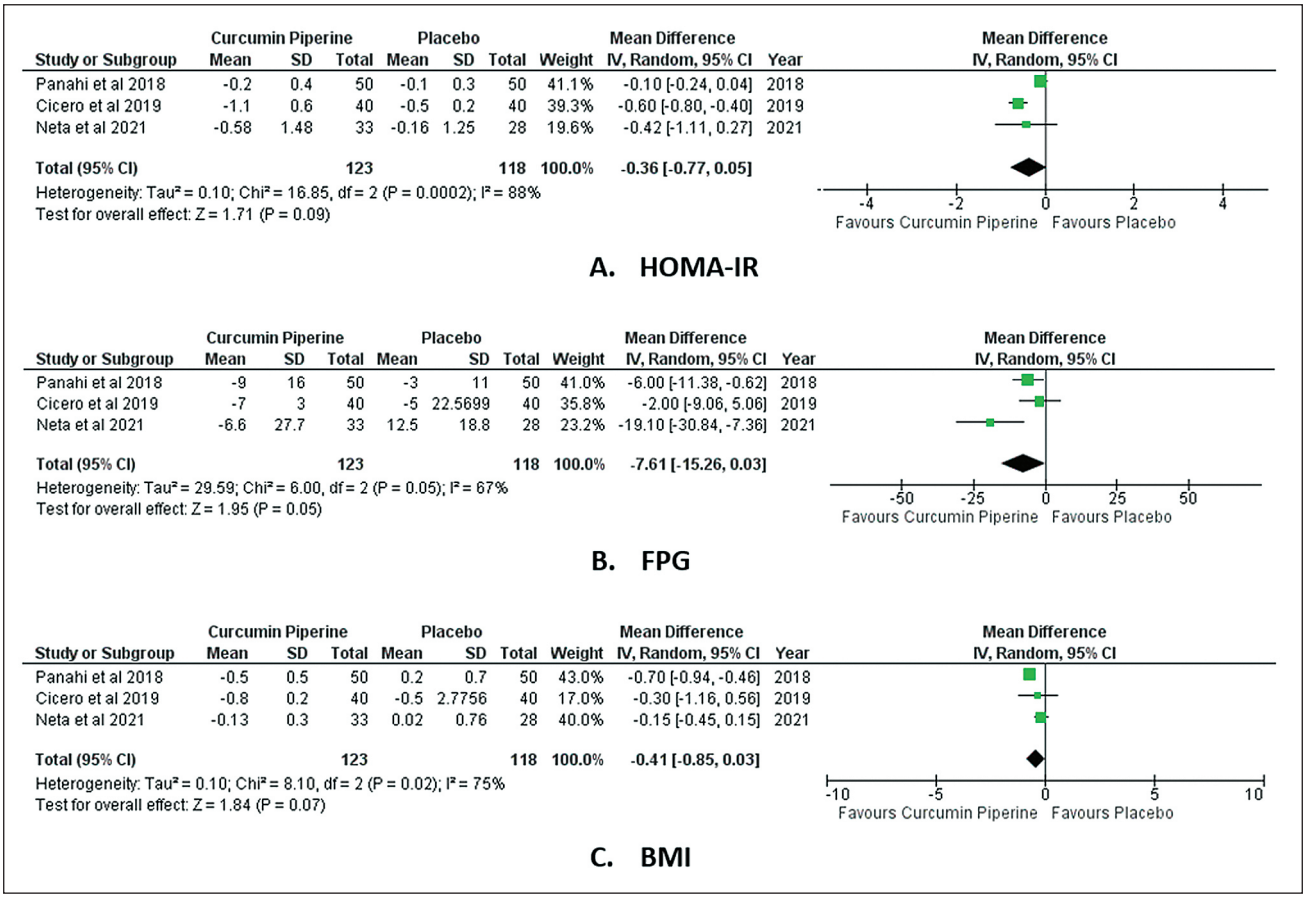

Figure 3. (A) Meta-analysis results [Forest plot] for Fasting Plasma Glucose (FPG); **(B)** Meta-analysis results [Forest plot] for Homeostatic Model Assessment for Insulin Resistance (HOMA-IR); and **(C)** Meta-analysis results [Forest plot] for Body Mass Index (BMI) in T2DM patients before and after supplementation of curcumin piperine capsule compared to placebo groups.

obese in all studies. Factors leading to a higher BMI include a sedentary lifestyle, alcohol consumption, low socioeconomic status, poor diet, age, sex and genetics.[23] A study by Kim et al. showed that women are more likely to become obese when compared to men.[24] Mosha et al. found that individuals aged 35 years and older were at the highest risk (69%) of becoming overweight and obese.[25] These findings are consistent with the characteristics observed in the study by Neta et al.,[18] which included a predominantly female and retired population who are known to be more sedentary. It also aligns with the average age reported in all three studies, ranging between 40 and 50 years. The HOMA-IR scores across the three studies are also relatively similar, showing significant insulin resistance. Among numerous factors that may contribute to the worsening of this parameter, BMI has the greatest impact.[26] The difference was observed solely in the FPG values, particularly in the study conducted by Neta et al.,[18] where it exhibited higher levels in the experimental group than in the control group. This variation occurred regardless of randomization protocol and despite a homogenous sociodemographic profile between the two groups. The FPG values are also distinct in the study by Cicero et al.,[17] since the prediabetes population was used. The inconsistencies identified may have an impact on the findings of the studies.

Curcumin is a hydrophobic polyphenol, an extract from the rhizome of Curcuma longa L. (turmeric), a species belonging to the Curcuma genus (Zingiberaceae family). The main constituents of curcumin are curcumin I (1,7-bis(4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione), curcumin II (demethoxycurcumin) and curcumin III (bisdemethoxycurcumin).[27] The three types of curcumin constituents differ in their aromatic rings. These three have been shown to exert anti-inflammatory and antioxidant effects, amongst other beneficial biological activities, including antitumoral, neuroprotective, antimicrobial, hepatoprotective and antirheumatic activities.[27]

T2DM patients with poor glycemic control can be characterized by their constant hyperglycemic state. The constant and chronic hyperglycemic state contributes to the production of Advanced Glycation End products (AGEs) and Reactive Oxidative Species (ROS).[28] These products will result in lipid peroxidation, an increase in oxidative stress (reinforced by the upregulation of MAPK, NADPHOX, NFKB, and TGFB), inflammation (caused by the upregulation of NFKB, TNF-a, and IL-6), VEGF, ICAM-1, VCAM-1, endothelial dysfunction and cellular apoptosis. These processes increase the risk of microvascular and macrovascular complications.[8]

In T2DM, hyperglycemia and hyperinsulinemia occur secondary to disruptions in beta cell function and insulin resistance. The increase in lipid peroxidation, hyperglycemia and increased hepatic glucose production promotes lipotoxicity, glucotoxicity and glucolipotoxicity, respectively, leading to the activation of pro-apoptotic signals to the pancreatic beta-cells.[28] This event amplifies the insulin-resistant state of the multiple organs caused by the mitochondrial dysfunction. As the cells continuously become more resistant to insulin, a decrease in beta-cell function can also be expected. These processes lead to the progression of T2DM.

The antidiabetic properties of curcumin can be attributed to the reduction of hepatic glucose production by activating AMP kinase and inhibiting both glucose-6-phosphatase and phosphoenolpyruvate carboxykinase activity.[16] This leads to the reduction in glucolipotoxicity, therefore reducing oxidative stress. Not only that, but curcumin also exerts unique biological properties. The active compound of Curcuma produces significant immunosuppressants that inhibit the production of IL-2 and IL-12.It also inhibits iNOS (inducible nitric oxide synthase), COX-2 (cyclooxygenase-2), lipoxygenase-5 and other pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, and IL-8.[8]

The anti-apoptotic characteristic of curcuminoids can be seen, as neurotoxic factors in macrophages and alveolar monocytes stimulated by lipopolysaccharides were also suppressed. It inhibits phosphorylation and degradation of the nuclear factor of kappa light polypeptide gene enhancer in B cells and activates the gamma receptor mechanism activated by the peroxisome proliferator.[8] This, in turn, reduces overall inflammation induced by the NF-kB pathway. The anti-inflammatory and anti-apoptotic effects will assist in the preservation of the beta-cell functions, together with the reduction of glycemic parameters and improvement in the HOMA-IR, therefore reducing the progression of T2DM.[8]

Researchers have yet to find an effective dose of curcumin.[35] However, many have hypothesized that it has a dose-dependent effect, producing greater benefits at a higher dosage.[8,36] In animal and human studies, curcumin has been shown to have good tolerability and safety, even at high doses (up to 12 g/day orally). Curcumin is to be taken with precaution and is not recommended for pregnant or lactating women, children, or those with anemia or liver disease.[29] Despite the wide range of beneficial effects, curcumin presents with the downside of having low solubility (in water), degradation in alkaline environments, crystallization in acidic environments and rapid metabolism (glucuronidation and sulfation in the liver and plasma, converting it into water-soluble metabolites to be excreted in the urine). These characteristics decrease its absorption in the gastrointestinal tract, translating to low bioavailability.[8]

Piperine is an alkaloid mainly found in *P. nigrum* (Black Pepper). The chemical structure of piperine is *N-piperoylpiperidin; (E, E)-1-(5-[1,3-benzodioxol-5-yl] -1-oxo-2,4-pentadienyl)- piperidine*. Many studies have shown that piperine increases the bioavailability of many drugs by promoting rapid absorption of drugs and nutrients or by inhibiting several cytochrome P450 enzymes and

phase II reactions.[30] Piperine has been shown to increase solubility, plasma levels, improve the pharmacokinetic profile and cellular absorption of curcumin.[31] Shiba et al., showed that the administration of 2 g of curcumin together with 20 mg of piperine to healthy individuals increased the bioavailability of curcumin by 2000 % with no adverse effects.[32] The study by Panahi et al., showed a significant reduction in serum levels of C-peptide, HbA1c and glucose in the curcumin-piperine-treated group versus the placebo group.[16] These studies showed the synergistic effect of curcumin and piperine, especially on glycaemic, inflammatory and hepatic markers in T2DM patients.[29]

Meta-analysis was conducted and the outcome was presented as mean and standard deviation. The meta-analysis showed no significant difference (p = 0.05) in FPG between the curcumin-piperine and placebo groups, with a mean difference of 7.61, favoring the curcumin-piperine group. An *in vivo* study done by Kaur et al., on diabetic rats showed significantly decreased plasma glucose levels (p<0.001) treated with curcumin with piperine and quercetin (extracted from *Allium cepa*) for four weeks.[33]

The meta-analysis for HOMA-IR and BMI also showed insignificant differences (p = 0.07 and p=0.09, respectively) for the curcumin/piperine group compared to the placebo group. These results are in accordance with another study performed in Iran by Hodaei et al.,[34] which found that oral supplementation of curcumin could reduce the levels of inflammatory biomarkers, but not insulin resistance markers such as HOMA-IR. The author hypothesized that it may be due to the short duration of intervention in both groups. In this review, we also found that duration of interventions is varied between studies, with Neta et al.,[18] being the longest (120 days) and Cicero et al., as the shortest (60 days);[17] the other is Panahi et al (90 days).[16] Regarding the BMI results, the lack of association may be due to the failure to adjust for body weight at the beginning of the study, therefore the baseline values were not similar. The limited number and high heterogeneity of these studies could have caused the lack of significant results for this meta-analysis.

Strength and limitation of the study

This study provided a comprehensive systematic review and meta-analysis of the effect of the combination of curcumin and piperine on the glycemic profile in patients with prediabetes and T2DM. However, this systematic review still has several limitations. Two studies from Panahi et al.,[16] and Neta et al.,[18] included T2DM patients who take standard anti-diabetic medications as their study participants. Neta et al., excluded patients who consume insulin as antidiabetic treatment.[18] Cicero et al., administered a higher dosage of the interventions compared to the other two studies.[17] Furthermore, they included prediabetic patients and excluded patients who are on oral or injectable medications for diabetes. The lack of studies was a significant limitation that may have affected the results. Language bias was inevitable as these studies were written in languages other than English, leading to other types of bias, as the number of studies did not fulfill the requirement for a quantitative publication bias analysis using a funnel plot. Further studies, particularly those with clinical significance, must be conducted.

CONCLUSION

In summary, the supplementation of curcumin and piperine showed a trend toward reducing FPG, HOMA-IR and BMI, but the results were not statistically significant. Further research is needed as there is a paucity of studies included in the review.

Acknowledgments
The authors would like to express their gratitude to all their colleagues from Atma Jaya Catholic University of Indonesia for all their support.

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

CRediT Author Statement
NDW: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **ET:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Project administration; **LFJJ:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft preparation, Writing – review and editing; **SA:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Project administration; **ESA:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft preparation, Writing – review and editing; **MRI:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft preparation, Writing – review and editing, Supervision.

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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CASE SERIES

Journal of the ASEAN Federation of Endocrine Societies

Primary Hyperparathyroidism during Pregnancy: Two Tales with Different Outcomes

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Abstract

Primary hyperparathyroidism (PHPT) is rare in pregnancy. This condition is challenging to diagnose and manage due to the limited diagnostic and therapeutic options that are safe during pregnancy. If not diagnosed and managed in a timely manner, serious maternal and foetal complications may occur. We report two cases, one with surgical intervention and one without, to show the importance of timely surgical intervention and discuss the challenges in the management of PHPT in pregnancy.

Key words: endocrine disorders in pregnancy, primary hyperparathyroidism, pregnancy, hypercalcaemia

INTRODUCTION

Primary hyperparathyroidism (PHPT) is a bone and mineral metabolism disorder caused by the autonomous secretion of parathyroid hormone (PTH). PHPT is rare in pregnancy, with a quoted incidence of less than 1% of all women with PHPT.[1,2] PHPT is caused by an increased secretion of parathyroid hormone (PTH) by one or more of the four parathyroid glands resulting in hypercalcaemia. Most commonly, PHPT is due to a parathyroid adenoma (85–95%), followed by parathyroid hyperplasia and rarely parathyroid carcinoma (less than 1%). PHPT poses serious complications for the mother and foetus. PHPT during pregnancy is challenging to diagnose and difficult to manage. The diagnosis of PHPT in pregnancy is often delayed due to the non-specific and subtle symptoms of the disorder, such as fatigue, nausea and vomiting which are frequently demised as symptoms commonly present during pregnancy. Moreover, as opposed to diagnosing PHPT outside of pregnancy where a battery of tests can be done including imaging involving ionising radiation and nuclear isotopes, the safest imaging for diagnosing PHPT in pregnancy is confined to an ultrasound of the neck which has a lower yield. Furthermore, many therapeutic options for the medical management of PHPT are not recommended in pregnancy, making parathyroidectomy the standard-of-care approach in the treatment of PHPT including PHPT during pregnancy.[3]

Prior to the recent expert consensus released by the European Society of Endocrinology (ESE) in 2022 which remains the only well-recognised international consensus,[4] there were no guidelines or consensus on the management of PHPT during pregnancy that could be widely implemented globally. The ESE expert consensus recommended parathyroidectomy in the second trimester of pregnancy for pregnant women with PHPT and serum-corrected calcium levels >2.85 mmol/L and/or >0.25 mmol/L above ULN and/or an ionised calcium >1.45 mmol/L.[4] For calcium levels below the above-mentioned thresholds, therapeutic options of surgery versus conservative management should be offered to the patient, and a shared decision should be made.[4]

We present two cases of PHPT diagnosed in pregnancy, one with a favourable maternal and foetal outcome after a timely parathyroidectomy and another with a fatal foetal complication when surgery is refused. Through the two cases described below, the authors would like to show the importance of timely surgical intervention in the management of PHPT during pregnancy. The authors emphasize the importance of having a high index of suspicion for serious diseases and the need for further investigations when there is an atypical presentation of symptoms. The management described in both cases adheres to the ESE consensus.

CASES

Case 1

A 35-year-old female in her third pregnancy presented at 27 weeks of gestation with prolonged nausea and vomiting up to her second trimester of pregnancy. There was no

eISSN 2308-118x (Online)
Printed in the Philippines

Received: June 28, 2023. Accepted: August 21, 2023.
Published online first: February 8, 2024.
https://doi.org/10.15605/jafes.039.01.17

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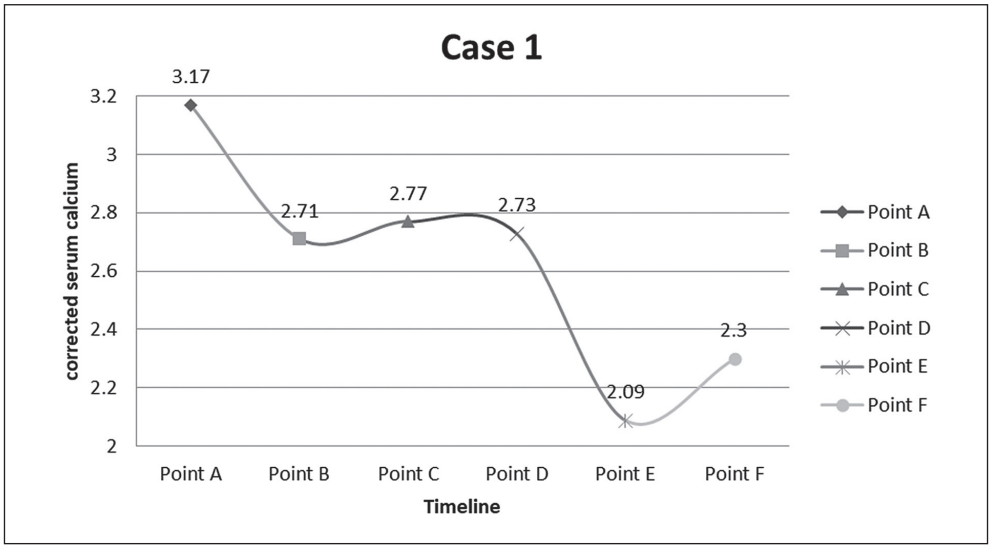

Figure 1. Serial corrected serum calcium levels for Case 1.

Point A: Upon initial admission; Point B: After inpatient intravenous rehydration with forced diuresis; Point C: After outpatient oral rehydration; Point D: Upon elective admission for parathyroidectomy; Point E: Post parathyroidectomy (when not requiring IV Calcium infusion); Point F: Upon outpatient follow-up (more than 2 months post parathyroidectomy).

history of miscarriage. Given the atypically prolonged symptoms, further workup was done. Blood results showed a raised corrected serum calcium of 3.17 mmol/L (reference range: 2.20-2.65 mmol/L) and normal renal function. Other blood investigations are shown in Table 1. Ultrasound of the neck showed an enlarged left superior parathyroid gland measuring 8 x 9 x 19 mm. She was admitted to the ward, and her corrected serum calcium levels reduced to 2.71 mmol/L after 7 days of intravenous rehydration with forced diuresis. She requested discharge against medical advice because of home commitments. Repeated corrected serum calcium increased after a 1-week trial of outpatient oral rehydration (Figure 1). Given failed conservative therapy and the huge adverse implications of hypercalcemia on the mother and foetus, a multi-disciplinary discussion and a family conference were conducted. A decision was reached to perform a left superior parathyroidectomy, which was done at 29 weeks of gestation. Histopathological examination was consistent with a left parathyroid adenoma. Her serum calcium returned to normal following parathyroidectomy, and her nausea and vomiting resolved. Subsequently, she presented at 38 weeks of gestation in the latent phase of labour and an emergency caesarean section was done for poor progress of labour. She delivered a healthy male infant with a birth weight of 3.35 kg.

Case 2

A 39-year-old female in her fifth pregnancy presented at 19 weeks of gestation with a recurrent urinary tract infection (UTI). There was no history of miscarriage. Given recurrent UTI in pregnancy, further investigations were done to try to ascertain the cause. Blood results during admission revealed corrected serum calcium of 2.87 mmol/L and normal renal function. Other blood investigations are shown in Table 1. Ultrasound of the neck showed an enlarged left inferior parathyroid gland measuring 17 x 12 x 22 mm and multiple American College of Radiology Thyroid Imaging Reporting and Data System (ACR TI-RADS) category 4 thyroid nodules. After 9 days of inpatient intravenous rehydration with forced diuresis, repeated corrected serum calcium normalized (Figure 1). Parathyroidectomy with hemithyroidectomy was advised after fine needle aspiration cytology (FNAC) of thyroid lesions. She refused FNAC and was desirous for discharge, and refused any further intervention. She was advised to drink 3 litres of fluid per day. A follow-up appointment was scheduled 1 week later to decide on further management after a multi-disciplinary discussion. Six days after discharge, she noticed no foetal movement while at home and decided to seek medical attention. A diagnosis of intrauterine death was made, and she was admitted to the obstetric ward for termination of pregnancy. Her corrected serum calcium

Table 1. Other relevant blood investigations

	Serum phosphate (reference range 0.81-1.45 mmol/L)	Alkaline phosphatase (reference range 30-120 U/L)	Intact PTH (reference range 14.9-56.9 pmol/L)	25-Hydroxy Vitamin D (nmol/L)	Calcium clearance to creatinine clearance ratio*
Case 1	0.56	601	346.0	28.03	0.016
Case 2	0.67	121	139.4	17.74	0.013

PTH: Parathyroid hormone.
*Calculated using the Hammersmith urine calcium to creatinine ratio [urine calcium (mmol/L) × (serum creatinine (μmol/L)/1000 divided by serum calcium (mmol/L) × urine creatinine (mmol/L)].

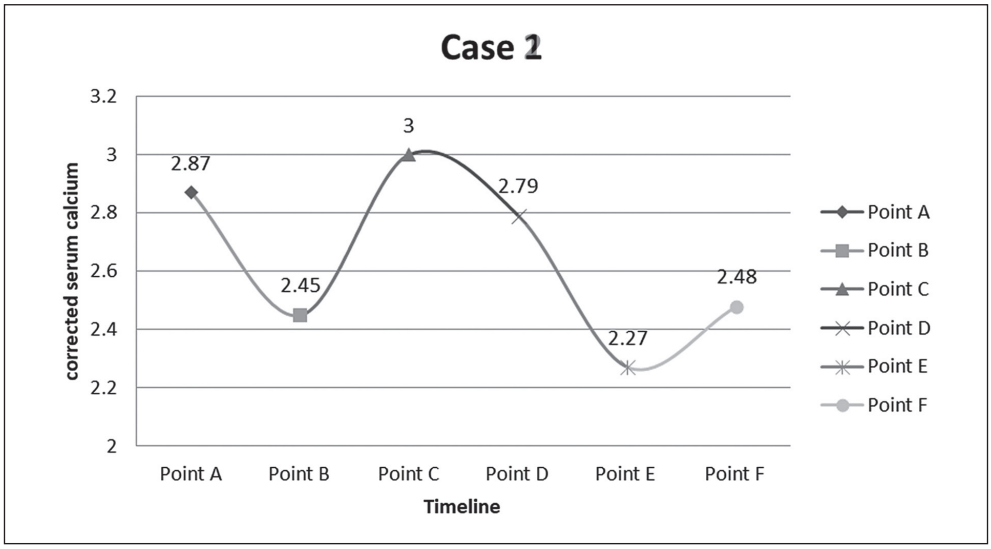

Figure 2. Serial corrected serum calcium levels for Case 2.

Point A: Upon initial admission; Point B: After inpatient intravenous rehydration with forced diuresis; Point C: After outpatient oral rehydration; Point D: Upon elective admission for parathyroidectomy; Point E: Post parathyroidectomy (when not requiring IV Calcium infusion); Point F: Upon outpatient follow-up (more than 2 months post parathyroidectomy).

was markedly raised on admission and normalised after 4 days of intravenous rehydration with forced dieresis and 1 dose of intravenous bisphosphonate (pamidronate). She was sad but aware of the risk of miscarriage, which was explained to her prior to being discharged home during the first admission, and she accepted the outcome. After the postpartum period, hemithyroidectomy and left inferior parathyroidectomy were done. Histopathological examination was consistent with a left inferior parathyroid adenoma and micropapillary carcinoma of the thyroid, and treatment was given accordingly.

DISCUSSION

PHPT in pregnancy presents either asymptomatically or with symptoms such as lethargy, hypertensive disorders in pregnancy, thirst, abdominal pain, constipation, nephrolithiasis, pancreatitis, hyperemesis gravidarum, and hypercalcaemic crisis. The symptoms mentioned above are very non-specific and some of them are frequently dismissed as symptoms commonly present during pregnancy, resulting in a delayed or missed diagnosis. Compared to other case reports, there was a delay in establishing the diagnosis of PHPT in both cases. Both patients were not symptomatic pre-pregnancy, were not known to have PHPT prior to pregnancy, and did not have significant past medical or family histories. Case 1 presented with non-specific symptoms of prolonged nausea and vomiting until late into the second trimester and the symptoms were dismissed as pregnancy symptoms, while Case 2 had an atypical presentation as she was asymptomatic but presented with a recurrent UTI that was not investigated before. Both cases had elevated serum calcium levels. A high level of clinical suspicion is required for further investigations to confirm the diagnosis of PHPT.

When left untreated, PHPT poses many serious complications to the mother and foetus ranging from hyperemesis gravidarum, hypercalcaemic crises in the mother, preterm delivery or miscarriage, to neonatal hypocalcaemia. Hence, PHPT should be given prompt and effective treatment.

Local practices and expert opinions still dictate a large part of the management of PHPT in pregnancy.[5] This is because prior to the recent expert consensus released by the European Society of Endocrinology in 2022,[4] there was a lack of guidelines or consensus on management of PHPT particularly during pregnancy. Moreover, there are no randomized control trials to guide management decisions for PHPT during pregnancy, and recommendations are based on limited evidence from observational studies and personal experience.[4] Parathyroidectomy is the only curative treatment for PHPT.[6] Advances in the effectiveness and safety of surgical techniques have added confidence to its recommendation,[7] and first-time cure rates have been reported to be over 95% in experienced hands.[8] Conservative treatment is viewed as a stopgap measure until surgery is performed.[4] Conservative treatment options are limited during pregnancy because many therapeutic options are not approved for use in pregnancy; management is mainly confined to oral and intravenous rehydration with or without forced diuresis. The use of medications such as cinacalcet,[9–12] calcitonin[12,13] and bisphosphonates[14,15] in PHPT during pregnancy has been reported but is not widely used due to insufficient data to recommend its wide usage, safety concerns in pregnancy, or lesser efficacy.

Prior to surgery, localization of abnormal parathyroid glands is needed. In both cases described, ultrasound of the neck was the only imaging modality used and was adequate to aid in establishing the diagnosis of PHPT. In cases where ultrasound of the neck fails to localise the lesion, contrast-

enhanced magnetic resonance imaging (MRI), ^{99m}Tc-methoxyisobutylisonitrile (^{99m}Tc-MIBI) scans, sestamibi single-photon emission computer tomography (SPECT/CT), 18F-Fluorocholine positron emission tomography (PET)/CT, or methionine PET/CT can be offered after weighing risks and benefits.[4]

There have been different opinions as to the indications and the best timing for performing parathyroidectomies in pregnant women. Unless contraindicated, international guidelines recommend surgery in PHPT patients under the age of 50.[7] The ESE expert consensus recommended parathyroidectomy in the second trimester of pregnancy for pregnant women with PHPT and serum-corrected calcium levels >2.85 mmol/L and/or >0.25 mmol/L above ULN and/or an ionised calcium >1.45 mmol/L[3,4] and all patients with symptomatic PHPT.[3] For calcium levels below the above-mentioned thresholds, therapeutic options of surgery versus conservative management should be offered to the patient, and a shared decision should be made.[4] It has been traditionally suggested that the second trimester of pregnancy is the best timing for parathyroidectomy, as surgery in the first trimester may involve a consequence of anaesthetic medications on incomplete organogenesis, while surgery in the third trimester may risk inducing preterm labour.[3,4] The patient's condition should be optimised and calcium levels brought down to normal prior to surgery, and the surgery does not necessarily need to be done during the same admission where the diagnosis was established. If the ideal timing of the second trimester is missed and conservative therapy fails, there are reports of third-trimester parathyroidectomy being performed safely.[16–18]

As to our management approach for both cases, intravenous rehydration and forced diuresis were initiated immediately in both cases, which successfully lowered the corrected serum calcium levels. However, corrected serum calcium levels remain elevated and refractory to outpatient oral rehydration. Other therapeutic options commonly prescribed in non-pregnant PHPT cases such as bisphosphonate,[14,15] cinacalcet,[9–12] and calcitonin,[12,13] were not prescribed for both cases as there are limited data for use in pregnancy. As a result, parathyroidectomy was planned for both cases, but only Case 1 had it done successfully during the third trimester of her pregnancy. After parathyroidectomy, her serum calcium returned to normal, her symptoms subsided, and her pregnancy was relatively uneventful, except for a caesarean section for obstetric indication. Unfortunately for Case 2, a delay in parathyroidectomy and conservative management with oral rehydration while awaiting for her decision for surgery resulted in suboptimal control of her serum calcium levels, as evidenced by an increasing serum calcium trend, eventually leading to an unwanted outcome—intrauterine death (IUD) in the second trimester of her pregnancy. The event of the IUD was attributed to hypercalcaemia as it coincided with the peak of the calcium level (3 mmol/L), and it is a known complication of hypercalcaemia beyond serum calcium levels of 2.85 mmol/L.[19] This could have been prevented with adequate inpatient intravenous hydration to bring the calcium levels down, followed by a parathyroidectomy.

CONCLUSION

Clinicians should have a high index of suspicion for PHPT in pregnancy and manage the condition with a multidisciplinary team consisting of an endocrinologist, an endocrine surgeon, an obstetrician, a paediatrician, and an anaesthesiologist promptly due to its potential serious maternal and foetal adverse outcomes if left untreated.

Acknowledgments
The authors thank the Director General of Health of Malaysia for his permission to publish this article.

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

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

CRediT Author Statement
YDL: Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – original draft preparation, Visualization, Project administration; **MHMA:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – review and editing, Supervision, Project administration

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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Journal of the ASEAN Federation of Endocrine Societies

CASE SERIES

Different Forms of Hypothyroidism in Infants with Maternal Graves' Disease: A Case Series

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Abstract

Infants of mothers with Graves' disease (GD) may develop central hypothyroidism (CH) due to exposure of the foetal hypothalamic-pituitary-thyroid axis to higher-than-normal thyroid hormone concentrations, primary hypothyroidism (PH) due to transplacental passage of maternal thyroid stimulating hormone receptor antibody (TRAb), antithyroid drugs (ATD) or thyroid dysgenesis secondary to maternal uncontrolled hyperthyroidism. We describe two infants with PH and four infants with CH born to mothers with poorly controlled Graves' disease. All infants required levothyroxine and had normal developmental milestones. While national guideline consensus for high thyroid stimulating hormone (TSH) on neonatal screening is well-established, thyroid function tests (TFTs) should be serially monitored in infants with low TSH on screening, as not all mothers with Graves' disease are diagnosed antenatally.

Key words: infants, central hypothyroidism, primary hypothyroidism, congenital hypothyroidism, maternal Graves' disease, maternal hyperthyroidism

INTRODUCTION

Graves' disease in a pregnant woman can lead to either hyper- or hypothyroidism in the foetus and neonate, depending on the control of hyperthyroidism, the presence of maternal stimulatory and/or inhibitory antibodies and antithyroid drug effects.[1-5] While hyperthyroidism in neonates of mothers with Graves' disease (GD) is well-described, hypothyroidism is less commonly reported.

Infants born to mothers with Graves' disease may develop central hypothyroidism (CH) or primary hypothyroidism (PH).[6-10] The most likely cause of central hypothyroidism is exposure of the foetal hypothalamic-pituitary-thyroid axis to higher-than-normal thyroid hormone concentrations impairing physiologic maturation during intrauterine life.[11] PH can be transient due to transplacental passage of maternal thyroid stimulating hormone receptor antibody (TRAb) or antithyroid drugs (ATD). However, if maternal hyperthyroidism is severe, with high transplacental passage of maternal free thyroxine (FT4), permanent thyroid dysgenesis may ensue.[7]

Hypothyroidism is the most common preventable cause of mental retardation in children.[12] Therefore, it is imperative to identify and treat them as early as possible. We describe six infants with hypothyroidism born to mothers with Graves' disease.

METHODOLOGY

A retrospective review was conducted on all infants referred to Paediatric Department Hospital Putrajaya for infants of mothers with Graves' disease between 1st January 2021 until 30th June 2022. Data was extracted from the electronic medical record (EMR) system and the referral letters from the referring physicians. Demographic profile and treatment course of mothers and infants were obtained. Additional details such as anthropometrics and blood chemistry were also extracted. Informed consent was obtained for each patient.

All samples were analysed with Beckman Coulter UniCel DxI 800 Access Immunoassay System with reference range for TSH (0.770-5.640) mU/L, FT4 (9.5-16.2) pmol/L and FT3 (4.3-6.8) pmol/L.

CASE

Primary hypothyroidism

Case 1

A live baby girl was born via lower segment caesarean section (LSCS) with birth weight of 2.67 kg. Her mother had Graves' disease (GD) antenatally and was maintained on carbimazole with highest dose of 10 mg once daily (OD). She was later switched to Propylthiouracil (PTU) in early

eISSN 2308-118x (Online)
Printed in the Philippines

Received: May 31, 2023. Accepted: July 10, 2023.
Published online first: January 5, 2024.
https://doi.org/10.15605/jafes.039.01.06

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pregnancy until delivery. Thyroid ultrasonography (USG) revealed multinodular goiter with no malignant changes. Maternal TRAb titre was 2.25 IU/L (normal <1.75 IU/L).

Cord thyroid stimulating hormone (TSH) was 3.5 mU/L. Thyroid function test (TFT) on day 9 of life revealed high TSH 56.2 mU/L (reference range 0.770-5.640 mU/L), low free thyroxine (FT4) 6.6 pmol/L (reference range 9.5-16.2 pmol/L), and low-normal free triiodothyronine (FT3) 4.3 pmol/L (reference range 4.3-6.8 pmol/L). TFT on day 18 of life showed high TSH 105.29 mU/L, low FT4 6.3 pmol/L and normal FT3 5.12 pmol/L. TRAb was <0.8 IU/L. She was started on L-thyroxine 55 mcg/m^2/day the following day. Her developmental milestones were appropriate. Weight was on the 10th centile and her length was between 25th to 50th centile on the Centers for Disease Control and Prevention (CDC) growth charts.

Case 2

A baby boy was born via LSCS with birth weight of 2.5 kg. His mother had gestational diabetes mellitus, diet-controlled and GD diagnosed in early pregnancy. She was started on PTU with highest dose of 100 mg BD and post-delivery on carbimazole 5 mg OD. Thyroid USG revealed a small cyst at left superior pole measures 0.9 x 0.2 cm. Mother's TRAb was 9.14 IU/L.

The infant had high cord TSH of 42.18 mU/L and cord FT4 of 12.3 pmol/L. TFT at day 4 of life revealed high TSH 96.55 mU/L and FT4 of 18.2 pmol/L. He was treated with L-thyroxine 49 mcg/m^2/day at day 7 of life. As per consensus guidelines for hypothyroidism in Malaysia,[13] if venous TSH >40 mIU/L, treatment should be started regardless of FT4 concentration. No TRab was taken. His developmental milestones were appropriate, and his weight and length were on the 10th centile on CDC growth charts.

Central hypothyroidism

Case 3

A baby girl was born term at 38 weeks via spontaneous vaginal delivery with birth weight of 3.41 kg. Mother had gestational diabetes mellitus and hyperthyroidism for 5 years prior to delivery. She was on carbimazole with highest dose of 30 mg per day throughout pregnancy and her TRAb was <0.3 IU/L.

Baby's cord TSH was 0.07 mU/L. At day 9 of life, TFT revealed normal TSH 3.385 mU/L and low FT4 <3.2 pmol/L. She was started on L-thyroxine 123 mcg/m^2/day. TRAb was <0.3 IU/L. Other blood investigations were within normal (random serum cortisol 170 nmol/l, random blood sugar 5.0 mmol/L, urea 3.4 mmol/L, sodium 136 mmol/L, potassium 4.5 mmol/L, insulin like growth factor-1 (IGF-1) 98.1 ng/ml [NV: 16-148 ng/ml] and prolactin 423 uU/ml [NV: 152-1520 uU/ml]).

Developmental milestones were appropriate. Weight and height were within the 50th centile on the CDC growth charts.

Case 4

A baby girl was born preterm at 35 weeks and 2 days with birth weight of 1.98 kg. Antenatally, mother was diagnosed with GD with ophthalmopathy. She underwent radioactive ablation therapy (RAI) twice with total cumulative dose of 50 millicurie (mCi). Last RAI was 1 year and 2 months prior to delivery. She was initially hypothyroid post-treatment, but she became hyperthyroid again preconception. She was on PTU in early pregnancy and was switched to carbimazole in the second trimester. She had serially high TRAb at 10 IU/L in the first trimester and 5.6 IU/L in the second trimester.

Cord TSH was <0.005 mU/L. TFT at day 5 of life revealed low TSH <0.005 mU/L and high FT4 64 pmol/L. Repeat TFT on day 7 of life revealed persistently suppressed TSH of 0.009 mU/L and high free T4 of 54.4 pmol/L.

She was treated with oral carbimazole 0.4 mg (0.2 mg/kg/dose) twice daily (BD) and oral propranolol 0.2 mg (0.1 mg/kg/dose) BD. She was weaned off carbimazole at day 45 of life when TFT revealed TSH 0.005 mU/L and FT4 16.2 pmol/L. However, she developed central hypothyroidism at day 66 of life when TFT revealed TSH 0.018 mU/L, FT4 9.1 pmol/L and FT3 4.9 pmol/L. She was started on L-thyroxine at 39 mcg/m^2/day the next day. TFT was only taken three weeks after cessation of carbimazole due to restrictions during the COVID-19 pandemic. No TRAb was taken. Developmental milestones were appropriate. Weight was below 10th centile and length was on 10th centile on the CDC growth charts.

Case 5

A baby boy was born large for gestational age via LSCS at 38 weeks and 1 day with birth weight of 4.21 kg. Mother was para 2 and diagnosed with hyperthyroidism post-delivery. She was hypertensive maintained on methyldopa and aspirin and has been experiencing palpitations for two months. TFT showed low TSH at <0.005 mU/L and high FT4 at 47.2 pmol/L. She was then started on carbimazole. Anti-thyroglobulin (ATG) was high at 307.2 U/ml (normal <1.0 U/ml), anti-thyroid peroxidase (ATPO) taken twice was elevated at 242.4 to 366 U/ml (normal <10.00 U/ml) and TRAb was 21.36 IU/L. Her thyroid USG had features suggestive of Graves' disease or thyroiditis.

Cord TSH was 0.01 mU/L. At day 28 of life, TSH was inappropriately low at 0.454 mU/L despite low FT4 at 7.5 pmol/L. ATG was 1.9 U/ml and ATPO was 42 U/ml. TRAb was <0.8 IU/L. He was started on L-thyroxine 96 mcg/m^2/day the next day. Other blood investigations were within normal (urea 2.0 mmol/L, sodium 136 mmol/L, potassium 4.8 mmol/L, creatinine 64 umol/L and random serum cortisol 364 nmol/L).

Developmental milestones were appropriate. Weight was at 90th centile and length was at 50th centile on the CDC growth charts.

Case 6

A baby girl was delivered via LSCS at 38 weeks and 3 days with a birth weight of 2.85 kg. Mother was para 1+1 and diagnosed with Graves' disease 3 months prior to pregnancy, initially presenting with tachycardia. Her TRAb was 23.45 IU/L, ATG 10.12 U/ml and ATPO 4.67 U/ml.

Baby's cord TSH was 2.28 mU/L. At day 4 of life, TSH was normal with high 2FT4 (48.7 pmol/L). TFT at day 10 revealed FT4 16.4 pmol/l with low TSH of 0.11 mU/L. Repeat TFT at day 36 of life revealed FT4 of 11.7 pmol/L and TSH of 1.52 mU/L. No other pituitary hormones were examined. The child was started on L-thyroxine 60 mcg/m^2/day that same day. Baby's TRAb was 6.13 IU/L, ATG <10 U/mL and Anti TPO 5.52 U/mL. The infant's weight and length were at 50th centile on the CDC growth charts, with appropriate developmental milestones noted.

DISCUSSION

Four mothers were diagnosed with Graves' disease prior to pregnancy – one during pregnancy and one post-delivery (Table 1). The mother of Case 4 was hypothyroid for 3 months after the second RAI treatment but became hyperthyroid 10 months before conception. The mother of Case 5 was hyperthyroid after delivery and was on carbimazole.

All mothers had elevated TRAb except the mother of Case 3. TRAb level in Case 3 may be undetectable due to prolonged ATD use, however, TRAb has been reported to have a poor correlation with disease activity.[14] Although four of the mothers were diagnosed prior to pregnancy, they had poorly controlled hyperthyroidism due to lack of compliance. At the end of the study, all mothers were on carbimazole.

All mothers employed mixed feeding except in Case 6 who was exclusively formula-fed prior to weaning. After weaning was started, all infants were on formula feeding. All mothers were from urban residences with presumably adequate dietary iodine intake.

There were four female and two male infants (Table 2). During follow-up, none of the infants had palpable goitres. Among the infants tested, only one had elevated TRAb while two were not tested (Table 3). Two infants developed primary hypothyroidism while four infants developed central hypothyroidism. Among the infants with central hypothyroidism, other pituitary hormones were normal for Case 3 and Case 5 while Case 4 and Case 6 were not tested. Two infants, (Cases 4 and 6) developed transient hyperthyroidism prior to central hypothyroidism at 2 months and 1 month of age, respectively. Case 4 required carbimazole treatment initially, while Case 6 did not require ATD. At 2 years old, both infants required levothyroxine.

Table 1. Endocrine profile of mothers with Graves' disease

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age	36	42	28	36	34	30
Parity	1	5	2	2	2	1
Graves' Diagnosis	2 years antepartum	pregnancy	5 years antepartum	5 years antepartum	postpartum	3 months antepartum
USG	Multinodular goitre	Cyst left superior pole	NA	Diffuse goitre with features of thyroiditis	Suspicious Graves' disease or Thyroiditis	NA
TSH Receptor Ab (normal <1.75 IU/L)	2.25	9.14	<0.3	10	21.36	23.45
ATG (normal <1 U/ml)	<0.9	Not done	320.4	21.9	307.2	Normal
ATPO (normal <10 U/ml)	307.0	Not done	266.3	36.4	366	Normal

TSH-Thyroid stimulating hormone, Ab-Antibody, NA-Not Available, ATG-Antithyroglobulin antibodies, ATPO-Anti-thyroid Peroxidase antibodies

Table 2. Demographic characteristics of hypothyroid infants born to mothers with Graves' Disease

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Gestation	38 weeks	38 weeks	38 weeks	35 weeks 2 days	38 weeks 1 day	38 weeks 3 days
Birth Weight (kg)	2.67	2.5	3.41	1.98	4.21	2.85
Ethnicity	Chinese	Chinese	Malay	Malay	Chinese	Malay
Gender	Female	Male	Female	Female	Male	Female

Table 3. Laboratory profile and treatment of hypothyroid infants born to mothers with Graves' disease

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Cord TSH (mU/L)	3.5	42.18	0.07	<0.005	0.01	2.28
Treatment initiation (day of life)	Day 19	Day 7	Day 9	Day 67	Day 29	Day 36
TSH at treatment (mU/L)	105.29	96.55	3.385	0.018	0.454	1.52
FT4 at treatment (pmol/L)	6.3	18.2	<3.2	9.1	7.8	11.7
TRAb	<0.8	NA	<0.3	NA	<0.8	6.13
L-thyroxine dosage (mcg/m^2/day)	55	49	123	39	96	60

TSH – Thyroid stimulating hormone, FT4 – Free thyroxine, TRAb – Thyroid stimulating hormone receptor antibody, L-Thyroxine – Levothyroxine

All infants attained normal TSH values (Figure 1) by day 28 of life and FT4 (Figure 2) by day 45 of life. Apart from Case 4, all other infants achieved normal FT4 values by day 27 of life.

All infants were followed up for at least 2 years except for Case 6 who was 12 months old at the end of the study. All infants had appropriate developmental milestones as per Paediatric Protocols for Malaysian Hospitals, 4th Edition, 2019.[15]

CONCLUSION

In our case series, all infants required levothyroxine at the end of the study. Developmental assessment was done for at least 1 year and all infants remained under follow-up. Re-evaluation with thyroid ultrasonography to determine permanent or transient hypothyroidism is done at or after 3 years of age when myelination in the central nervous system is complete.[13] At the end of the study none of the infants had reached 3 years of age.

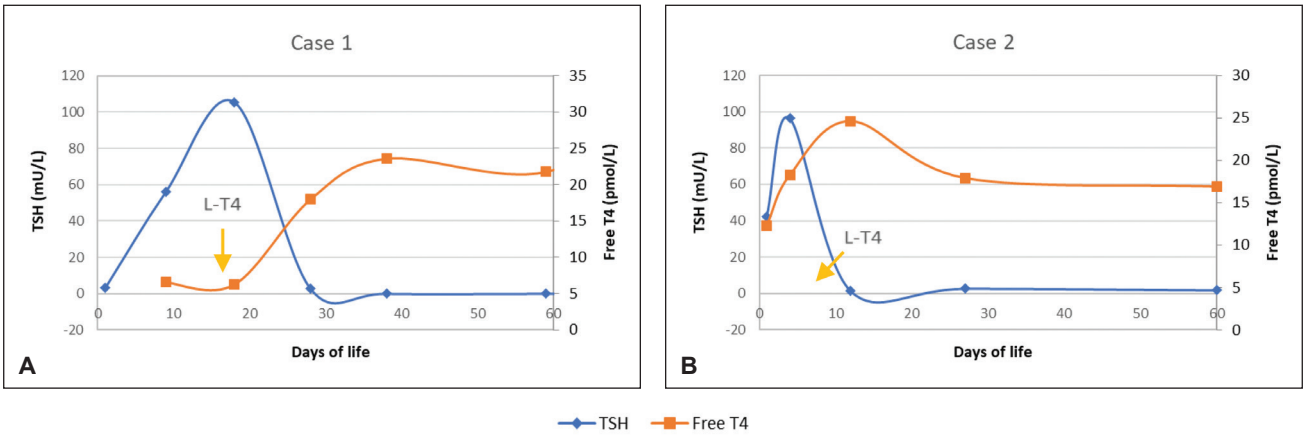

Figure 1. ***Primary hypothyroidism.*** **(A)** Case 1 and **(B)** Case 2 shows the start of levothyroxine (LT4) and corresponding changes of thyroid stimulating hormone (TSH) and free thyroxine (Free T4).

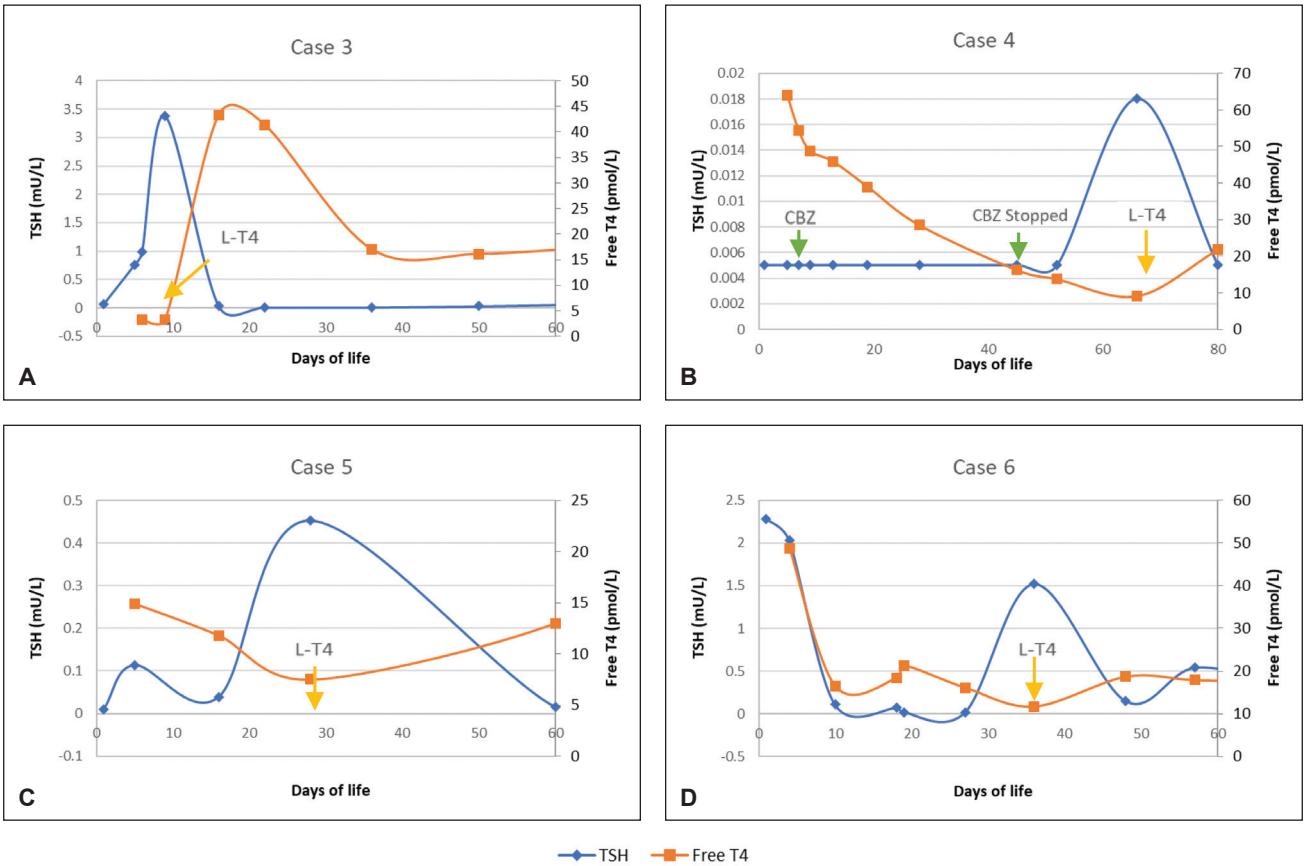

Figure 2. ***Central hypothyroidism.*** **(A)** Case 3, **(B)** Case 4, **(C)** Case 5 and **(D)** Case 6 shows start of levothyroxine and changes in TSH and FT4. Case 4 was started on carbimazole (CBZ) at day 7 of life and was weaned off CBZ at day 45 of life when TFT revealed TSH 0.005 mU/L and FT4 16.2 pmol/L. Case 4 was started on levothyroxine at Day 66 of life.

Infants of maternal Graves' disease (GD) should be monitored for both hyperthyroidism and hypothyroidism. Maternal gestational hyperthyroidism causes a hyperthyroid foetal environment due to increased thyroxine transfer which leads to suppression of the foetal hypothalamic-pituitary-thyroid axis and central hypothyroidism in newborns. Certainly, the most effective management would be the preservation of euthyroid status throughout pregnancy.[16] Primary hypothyroidism could be a result of transplacental passage of antithyroid drugs (ATD) during pregnancy, transplacental passage of maternal blocking antibodies or thyroid dysgenesis secondary to maternal hyperthyroidism.

While the Malaysia national guideline[13] consensus on re-evaluation for high TSH in neonatal screening is established, no recommendation exists for low TSH. As illustrated by our case series, the presentation of hypothyroidism in this group of patients is a spectrum and may be delayed. In view of this, it is imperative that TFT be serially monitored in infants with low TSH as not all mothers with Graves' disease are diagnosed antenatally.

Ethical Consideration
Patients' consent were obtained before submission of the manuscript.

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

CRediT Author Statement
AAL: Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing - original draft preparation, Writing - review and editing, Visualization, project administration; **JSLW:** Conceptualization, Methodology, Resources, Supervision, Project administration; **NS:** Conceptualization, Methodology, Resources, Supervision, Project administration; **JYHH:** Conceptualization, Methodology, Resources, Supervision, Project administration.

Authors Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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CASE REPORT

Journal of the ASEAN Federation of Endocrine Societies

Insulin Autoimmune Syndrome – An After-Meal Roller Coaster Ride

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Abstract

Hypoglycemic disorders are rare in persons without diabetes, and clinical evaluation to identify its etiology can be challenging. We present a case of insulin autoimmune syndrome induced by carbimazole in a middle-aged Chinese man with underlying Graves' disease, which was managed conservatively with a combination of dietary modification and alpha-glucosidase inhibitor.

Key words: hypoglycemia, endogenous hyperinsulinism, insulin antibodies

INTRODUCTION

Hypoglycemic disorders are rare in persons without diabetes, and clinical evaluation to identify its etiology can be challenging. In a seemingly well individual, the differential diagnosis of hyperinsulinemic hypoglycemic disorders involves two main categories: Accidental, surreptitious or even malicious hypoglycemia and endogenous hyperinsulinism.[1] The causes of endogenous hyperinsulinemic hypoglycemia include insulinoma, post-bariatric hypoglycemia, nesidioblastosis, and insulin autoimmune syndrome.[2] We present an uncommon case of insulin autoimmune syndrome induced by carbimazole in a middle-aged Chinese man with underlying Graves' disease, which was managed conservatively with a combination of dietary modification and alpha-glucosidase inhibitor.

CASE

A 57-year-old Chinese male was brought to the emergency department for syncope. He experienced palpitations, sweating and hand tremors before he passed out a few hours after finishing his meal. He denied chest pain or shortness of breath. There was no history of tongue biting or urinary incontinence. He had no fever or altered bowel habits. He reported unintentional weight loss of 5 kg in 2 months. His medical history was notable only for hyperthyroidism. He was prescribed carbimazole by a general practitioner. However, he took the medicine irregularly. He smoked and drank alcohol occasionally. He did not consume traditional remedies or recreational drugs. There was no family history of diabetes mellitus or malignancy. His sister also had a thyroid disorder.

His capillary blood glucose on arrival was 2.6 mmol/L. He regained consciousness promptly after being given intravenous glucose. His vital signs remained within normal limits. He did not appear septic or cachexic. He had no Cushingoid features or skin hyperpigmentation. Systemic examination was otherwise unremarkable.

In the ward, he continued having recurrent symptomatic hypoglycemia, which happened either a few hours after food intake or during the fasting state, with capillary blood glucose levels ranging between 2.3 and 2.4 mmol/L. He required an intravenous 10% glucose infusion and was prescribed frequent, small portions of a complex carbohydrate diet.

Upon further questioning, the patient recollected similar symptoms had occurred several times prior to this event. His symptoms could either happen a few hours after eating food or during an empty stomach.

His complete blood count, renal and liver function were within the normal range. Glycated hemoglobin was 5.8%. Thyroid-stimulating hormone (TSH) level was 0.01 mU/L [Normal value (NV): 0.48 – 4.17 mU/L] with free thyroxine (T4) level of 12.2 pmol/L (NV: 10.7 – 18.4 pmol/L). He had elevated thyroid autoantibodies with anti-thyroid stimulating hormone receptor (TSH) antibody level of 21.88 IU/L (NV: < 1.75 IU/L) and anti-thyroid peroxidase antibody (TPO) of 9870.78 IU/mL (NV: 0 – 9 IU/mL). Tumour markers were not detected. A short corticotropin stimulation test was performed and the result showed an adequate response with a peak cortisol level of 860.3 nmol/L at 60 minutes. A prolonged fasting test was arranged however it was terminated prematurely due to the patient's non-

eISSN 2308-118x (Online)
Printed in the Philippines

Received: May 12, 2023. Accepted: July 5, 2023.
Published online first: January 7, 2024.
https://doi.org/10.15605/jafes.039.01.07

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Table 1. Results of prolonged Oral Glucose Tolerance Test

Time (Hour)	Random plasma glucose (mmol/L)	Insulin (pmol/L) (NV: 17.8 – 173)	C-Peptide (pmol/L) (NV: 367 – 1467)
0	2.4	>6945	2506
1	9.7	>6945	4298
2	14.4	>6945	5763
3	13.6	>6945	5755
4	6.3	>6945	4773
5	1.5	>6945	3538
6	1.5	>6945	3212

adherence. A prolonged oral glucose tolerance test (OGTT) confirmed endogenous hyperinsulinemic hypoglycemia (Table 1). Serum β-hydroxybutyrate was not raised. Plasma proinsulin and blood for sulfonylurea were not offered by our laboratory. Computed tomography of the pancreas showed no pancreatic mass.

With the combination of dietary adjustment and commencement of acarbose 50 mg thrice daily, we managed to stabilize his plasma glucose. He was discharged well after 2 weeks of admission. Advice on dietary and medication adherence was re-emphasized, together with regular self-monitoring of blood sugar.

As the assay was not widely accessible, serum insulin autoantibody (IA) titer was only measured 6 months after his initial presentation. Serum IA titer was measured on a chemiluminescent immunoassay platform. The first IA measurement recorded high levels of 113.9 IU/mL (NV: <20.0 IU/mL), indicating the presence of IA in a non-diabetic patient without exposure to exogenous insulin. Carbimazole was restarted at a lower dose when the outpatient review of the thyroid function test revealed an elevated free thyroxine (T4) level of 39.4 pmol/L with a thyroid stimulating hormone (TSH) level of 0.01 mU/L. Repeat measurement of anti-TSH receptor antibody titer at 11 months after carbimazole treatment showed a reduction from 21.88 IU/L to 4.15 IU/L. His second measurement of serum IA titer also declined from 113.9 IU/mL to 88.51 IU/mL, as reflected by no new episodes of hypoglycemia while receiving carbimazole treatment.

DISCUSSION

Establishing Whipple's triad, which entails a low plasma glucose level (less than 3.0 mmol/L), the associated symptoms, and the resolution of symptoms after correction of the glucose level, forms the cornerstone in managing a subject with a hypoglycemic disorder.[1,3] Insulinoma was initially suspected as the cause of our patient's symptoms. Nonetheless, he had clinical clues that were atypical for insulinoma, including a history of weight loss, postprandial symptoms, and an extremely high insulin concentration above 1000 pmol/L, which is unusual for insulinoma or beta-cell hypertrophy.[3,4]

According to the Endocrine Society guideline published in 2009, insulin antibodies should be screened when endogenous hyperinsulinism is confirmed by plasma glucose of less than 3 mmol/L, serum insulin of at least 18 pmol/L, C-peptide of at least 200 pmol/L, and β-hydroxybutyrate of less than 2.7 mmol/L.[1] Yukimasa Hirata and colleagues first described Insulin autoimmune syndrome (IAS) or Hirata's disease in 1970, and it is now recognized as the third most common cause of spontaneous hypoglycemia in Japan following insulinoma and non-pancreatic neoplasia.[3] IAS is usually seen in adult patients older than age 40 years of age with equal gender distribution. It is characterized by autoimmune antibodies to endogenous insulin in individuals without previous exposure to exogenous insulin.[5]

IAS can present as recurrent fasting hypoglycemia, alternating with postprandial hyperglycemia.[6] This distinctive clinical picture had been conveniently demonstrated by the findings from our prolonged OGTT. The exact pathophysiology of hypoglycemia in IAS is unclear. It is hypothesized that there is an altered kinetics of insulin clearance, due to "buffering" by autoantibodies, which sequester insulin in immune complexes during the acute phase of insulin secretion, only to release it slowly later, at physiologically inappropriate times.[6] When insulin autoantibodies bind to insulin, the half-life of insulin becomes prolonged from minutes to hours, while the half-life of the C-peptide remains unaffected. This phenomenon leads to disproportionately elevated plasma insulin levels while having a non-elevated plasma C-peptide which skewed the insulin to C-peptide molar ratio to more than 1.[7] In our patient, the insulin to C-peptide ratio throughout the prolonged OGTT ranged from 1.2 to 2.7.

Insulin autoantibodies are typically polyclonal in origin and are mostly of the immunoglobulin G class. They can be of high affinity with low binding capacity or low affinity with high binding capacity. The latter is often associated with clinical manifestations. These antibodies are virtually indistinguishable from the antibodies seen in up to 70% of children with type 1 diabetes.[8]

The prevalence of IAS varies according to race. The presence of HLA DR4 allele has been demonstrated in 96% of Japanese patients with IAS, indicating a susceptible genetic background in this syndrome.[3,5] In the past decade, the number of reported cases among Whites has also increased.[4] IAS is also known to be associated with hematological disorders or autoimmune diseases.[3,5] On the other hand, insulin autoantibodies may be triggered by medications, or exposure to viruses or they may manifest spontaneously (Table 2).[3,5,7]

The list of medications associated with IAS is extensive. The interaction of drugs containing the sulfhydryl group with the disulfide bond in the insulin molecule has been postulated to play a role. Antithyroid drugs such as methimazole and supplements such as alpha-lipoic acid are the most frequently prescribed medicines associated with IAS, followed by other groups of medication.[3,5,7] The

Table 2. Trigger factors and diseases associated with Insulin Autoimmune Syndrome[3,4,7]

Trigger Factors and Associated Diseases	Examples
Genetic predisposition	HLA-DR4
Autoimmune disorders	Graves' disease, rheumatoid arthritis, systemic lupus erythematosus
Hematological disorders	Multiple myeloma, monoclonal gammopathy of undetermined significance
Medications	
Antithyroid drugs	Methimazole, carbimazole, propylthiouracil
Supplements	Alpha-lipoic acid, glutathione
Antihypertensives	Captopril, hydralazine, procainamide
Antiplatelet drugs	Clopidogrel
Antibiotics	Penicillamine, imipenem, isoniazid
Anti-inflammatory drugs	Steroids, diclofenac
Proton pump inhibitors	Pantoprazole, omeprazole
Plasma proteins	Albumin
Viruses	Measles virus, mumps virus, rubella virus, varicella zoster virus, coxsackie B virus, hepatitis C virus

onset time of drug-induced IAS differs greatly, from days to months and even years after the drug exposure. On average, the onset time is 4 to 6 weeks.[7] IAS is usually a transient condition in Japanese patients, with spontaneous resolution of up to 80% within 3 to 6 months of diagnosis reported.[4,11] Meanwhile, in non-Asian patients, symptoms generally improve, and resolve completely over time if patients stop taking the drug that caused the symptoms.[5] Then again, there are scattered case reports and series which highlight that persistent recurrent hypoglycemia in IAS can last for years.[6,7]

At present, there are no guidelines available for the management of IAS. Trial of dietary modification is routinely advocated as the first line of treatment, followed by pharmacological interventions in those who fail to respond.[3-6] After the withdrawal of the culprit drug, the dietary recommendations include eating small frequent meals, avoiding simple carbohydrates, and a high-fiber diet. The rationale for this approach is to prevent postprandial hyperglycemia. Glycemic excursions after meals determine the amount of insulin released from the beta cells; the higher the excursion, the greater the insulin secreted and bound to the antibodies for future release.[9,10]

In our patient, due to limited drug access and safety concerns, we adopted a combination of dietary adjustment and acarbose to curb persistent hypoglycemia. Acarbose acts by inhibiting the membrane-bound intestinal alpha-glucosidase which hydrolyzes oligosaccharides, tri-saccharides, and disaccharides to glucose and other monosaccharides in the brush border of the small intestine. This intervention staggers the glucose absorption and the postprandial glycemic excursions, thus reducing the quantum of insulin synthesis and secretion from the pancreatic β cells.[9] Other available drug therapies that can help to reduce pancreatic insulin secretion include somatostatin analogues and diazoxide.

Besides targeting endogenous insulin production, immune-modulating agents such as glucocorticoids, azathioprine, and rituximab have been used to decrease insulin autoantibodies levels.[3,5,9] In refractory cases, plasmapheresis or even pancreatic surgery have been tried by some investigators, with varying degrees of therapeutic success.[2,4,9-11]

Based on the literature review, IAS is largely a benign and self-limiting disorder. The recurrence rate of IAS after its full resolution is low.[3,5] Disease recurrence owing to the re-administration of the culprit drug only happens in a minority of patients. Our patient did not experience new hypoglycemic events when carbimazole was restarted at a lower dose. His insulin autoantibody titer also declined following the reduction of anti-TSH receptor antibody with carbimazole treatment.

CONCLUSION

In summary, our case highlights the importance of a structured diagnostic approach to spontaneous hypoglycemia. High insulin concentration along with insulin/c-peptide molar ratio of more than 1 should raise the clinical suspicion of IAS and is confirmed by the presence of high-titer insulin autoantibodies. Early recognition of this syndrome can avoid the need for laborious and costly investigations of presumed insulinoma.

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

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

CRediT Author Statement
CKL: Writing - original draft preparation; **HCW:** Writing - review and editing; **SA:** Writing - review and editing; **SLY:** Writing - review and editing

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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Uric Acid Crystalluria following the Recovery Phase of Diabetic Ketoacidosis (DKA): A Lesser-known Complication of DKA

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Key words: crystalluria, uric acid, diabetic ketoacidosis

The occurrence of hyperuricemia is frequently associated with diabetic ketoacidosis (DKA),[1] however, crystalluria from the precipitation of calcium oxalate, uric acid, or urate crystals, is less known. Metabolic derangements during DKA, especially acidic urinary pH and hyperuricosuria are the main risk factors for uric acid crystals and stones.[2] Here we report a case of uric acid crystalluria following the recovery phase of DKA.

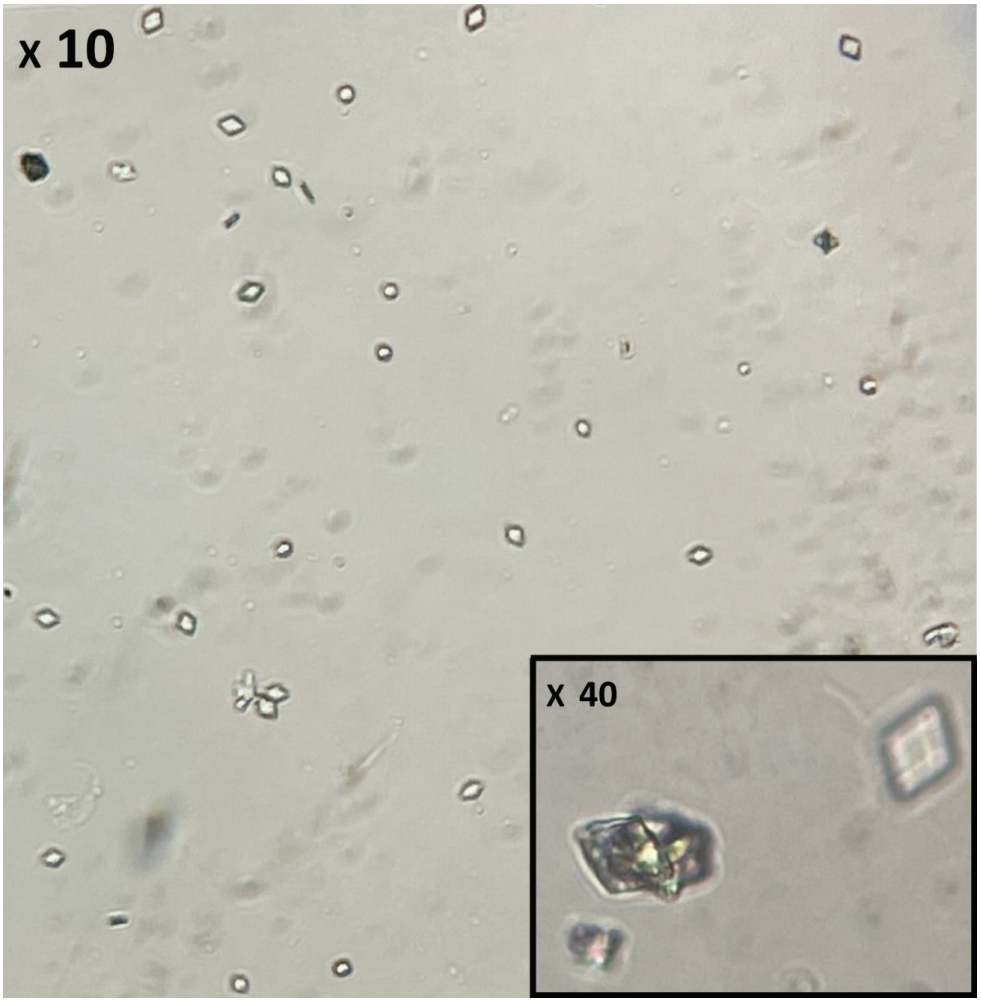

Figure 1. Urine sediment displays different types of shapes: barrel, plate-like, or diamond, consistent with uric acid crystals (x10); Diamond and rhomboid-shaped uric acid crystals at a higher magnification (x40).

eISSN 2308-118x (Online)
Printed in the Philippines

Received: December 11, 2023. Accepted: January 13, 2024.
Published online first: May 5, 2024.
https://doi.org/10.15605/jafes.039.01.20

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A 72-year-old male with persistent poorly controlled Type 2 DM was admitted due to COVID-19 pneumonia and severe DKA. On admission, his baseline serum creatinine increased from 1.3 mg/dL to 2.1 mg/dL. After intravenous insulin infusion and hydration, DKA was resolved after 15 hours with improved renal function to baseline. After DKA resolution, urinalysis showed an incidental finding of uric acid crystal particles with an acidic urine pH of 5.0. The concurrent level of plasma uric acid was within normal (6.6 mg/dL). Plain abdominal CT revealed no stones in the renal medulla or ureters. However, earlier blood samples on admission showed markedly elevated plasma uric acid levels (12.1 mg/dL). Further investigations revealed increased fractional excretion of uric acid from 7.4% at admission to 15.7% on the second day, indicating hyperuricosuria. With adequate diuresis and supportive treatment, crystalluria disappeared within 48 hours and he was discharged after 10 days. Our case highlights the importance of urine microscopy examination in patients with severe DKA to detect crystalluria which might contribute to renal impairment or nephrolithiasis following the recovery phase of DKA if left unchecked.[3] Clinicians should consider hyperuricemia which could lead to uric acid nephropathy from kidney stone as a late complication of DKA.

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

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

CRediTAuthor Statement
YT: Conceptualization, Methodology, Validation, Data curation, Writing – original draft preparation, Visualization, Funding acquisition; **NT:** Investigation, Project administration; **WK:** Software, Resources, Writing – review and editing, Supervision.

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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Deceptive Brown Adipose Tissue

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Key words: brown adipose tissue, pheochromocytoma, FDG-PET/ CT, Ga-68 DOTANOC PET/CT

A 23-year-old female presented with headache, palpitation, and hypertensive spells. There was no similar family history. Twenty-four (24) hour urine testing showed elevated normetanephrine level with normal metanephrines [metanephrines 123 mcg/24 hrs (74-297); normetanephrines 5321.16 mcg/24 hrs (73-808)]. A biochemical diagnosis of normetanephrine-secreting pheochromocytoma was made. Considering the age and urine reports, a functional scan was ordered. Imaging with 18-FDG PET CT was done which showed uptake indicative of a large left adrenal mass, as well as uptake in the mediastinal, abdominopelvic, lymph nodes and metabolically active mesenteric, peritoneal and omental thickness. This suggested a left adrenal pheochromocytoma with the possibility of an associated lymphoproliferative disorder or active lesions in brown fat (Figure 1A). To describe these extra-adrenal lesions, a Ga-68

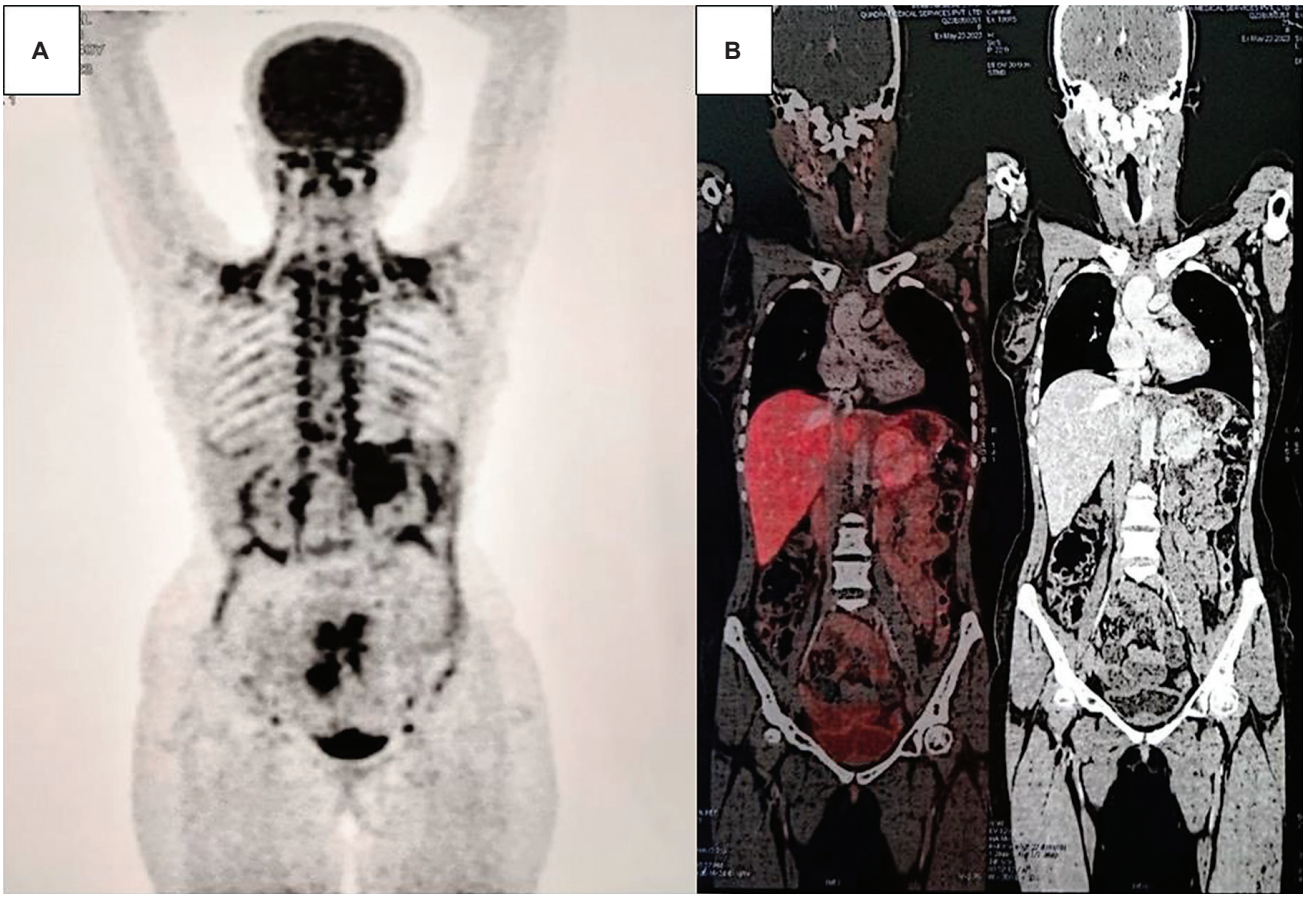

Figure 1. (A) 18-FDG PET CT done showed a large left adrenal mass, mediastinal, abdominopelvic lymph nodes, metabolically active mesenteric, peritoneal, and omental thickness suggestive of brown fat uptake; **(B)** Ga-68 DOTANOC PET CT showing a diffuse somatostatin receptor-expressing large soft tissue mass lesion in left adrenal likely pheochromocytoma.

eISSN 2308-118x (Online)
Printed in the Philippines

Received: September 5, 2023. Accepted: October 11, 2023.
Published online first: May 12, 2024.
https://doi.org/10.15605/jafes.039.01.21

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DOTANOC PET CT was obtained which showed a diffuse somatostatin receptor-expressing large soft tissue mass lesion in the left adrenal likely to be pheochromocytoma without any other lesion elsewhere in the whole body survey (Figure 1B).

This depicts the confusion created by the metabolically active brown adipose tissue (BAT) in the FDG PET scan. Brown fat is involved in non-shivering thermogenesis and is typically located in the cervical, supraclavicular, mediastinal, and abdominal regions. High uptake in the BAT can make interpretation of the FDG PET report difficult and misleading.[1] Some precautions like avoidance of cold and beta blockers can minimize BAT uptake in FDG-PET scans.

Although it has been found in the literature that even Ga-68 DOTANOC PET CT scan can show BAT uptake,[2] it is far less frequent than with FDG-PET scan. Therefore Ga-68 DOTANOC PET CT should be used for functional imaging of Pheochromocytoma and Paragangliomas (PPGL).[3]

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

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

CRediT Author Statement
BP: Conceptualization, Software, Validation, Investigation, Resources, Writing – original draft preparation, Visualization, Project administration; **AD:** Software, Formal analysis, Resources, Writing – review and editing, Visualization; **PS:** Conceptualization, Methodology, Software, Validation, Investigation, Data curation, Writing – review and editing, Supervision; **AK:** Conceptualization, Methodology, Validation, Investigation, Data curation, Writing – review and editing; **PK:** Software, Formal analysis, Resources, Writing – original draft preparation, Supervision.

Author Disclosure
The authors declared no conflict of interest.

Funding Source
None.

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CECILIA A. JIMENO | Vice Editor-in-Chief
Prof. Jimeno receives honoraria for lectures from Glaxo Smith Kline, Sanofi Aventis, Menarini, Merck and Novo Nordisk. She receives travel grants for CME from Corbridge and Sanofi Aventis.

GABRIEL V. JASUL JR. | Associate Editor
Prof. Jasul received honoraria for lectures from Sanofi Aventis, Novo Nordisk, MSD, Boehringer Ingelheim, Abbott Nutrition, AstraZeneca, LRI- Therapharma, UMed and Woerwag.

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Prof. Mustafa receives grants from Novo Nordisk. She receives honoraria for lectures from Astra Zeneca, Novo Nordisk, Zuellig Pharma and Abbott. She receives support for attending meetings from Novo Nordisk and Sanofi. She is an Advisory Board member of Astra Zeneca and Novo Nordisk. She is a Board of Trustee of the National Diabetes Institute (NADI) and a Council Member of the Malaysian Endocrine and Metabolic Society (MEMS).

THAN THAN AYE | Associate Editor
Prof. Aye has nothing to disclose.

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Prof. Deerochanawong has nothing to disclose.

NGUYEN THY KHUE | Associate Editor
Prof. Khue receives consulting fees from Merck. She receives honoraria for lectures from Servier, Boehringer Ingelheim and Novo Nordisk. She receives fees as the Advisory Board of Kowa. She is a member of the Vietnamese Association of Diabetes and Endocrinology (VADE), Ho Chi Minh City Association of Diabetes and Endocrinology (HADE) and Ho Chi Minh City Medical Association.

MARY ANN R. ABACAN | Editorial Board Member
Prof. Abacan receives grants as co-investigator of the Registry of Lysosomal Storage Disorders from Sanofi Genzyme (2014 to present) and as co-investigator of the SHP-ELA-401 - A Long-Term, Open-Label, Multicenter, Phase IV Study to Assess Longitudinal Changes on Height and Weight in Patients with MPS II who are receiving Elaprase and started Treatment with Elaprase at <6 Years of Age - Shire (2015-present). She is a Consultant Medical Specialist at the Institute of Human Genetics at the University of the Philippines-National Institutes of Health. She received honoraria as a lecturer of the Training Workshop for Dietitians (Overview of Urea Cycle Disorders). She is a co-chair of the National Transplant Ethics Committee of the Department of Health and the Chair of the Metabolic Experts Panel at the Newborn Screening Reference Center of the National Institutes of Health. She is a stockholder of Clinical Genetics and Genomics Counselling Care Services, Inc. She is the Chair of the Congenital and Developmental Disorders Task Force, Philippine Periodic Health Examination II - Institute of Clinical Epidemiology and DOH joint project (2021), Technical Facilitator of the Pediatric Immunization Task Force, Philippine Periodic Health Examination III - Institute of Clinical Epidemiology and DOH joint project (2023) and a Technical Facilitator of the Dengue Task Force, Philippine Periodic Health Examination III - Institute of Clinical Epidemiology and DOH joint project (2023).

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Prof. Abad has nothing to disclose.

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Prof. Alejandria has nothing to disclose.

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Prof. Bagamasbad received grant funding from the Department of Science and Technology-Philippine Council for Health Research Development (DOST-PCHRD) to conduct research on prostate cancer and SARS-CoV-2 and the DOST-National Academy of Science and Technology (NAST) to conduct research on prostate cancer. She received honoraria as a plenary speaker of the Philippine College of Endocrinology, Diabetes and Metabolism (PCEDM) and the Philippine Society of Medical Oncology (PSMO). She is a member of the Endocrine (ENDO) Society (no financial involvement).

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Prof. Nattapol has nothing to disclose.

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Dr. Chng has nothing to disclose.

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Prof. Latt has nothing to disclose.

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Dr. Tangco has nothing to disclose.

NGUYEN VAN TUAN | Editorial Board Member
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Prof. Win has nothing to disclose.

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Dr. Reyes received a research grant to the Women's Health Care Foundation from the Philippine Council for Health Research and Development. She receives honoraria as a Technical Reviewer of research proposals and as a trainer on research ethics from the Department of Science and Technology-Philippine Council for Health Research Development (DOST-PCHRD). She was also the President and Chair of the Board of Trustees of the Women's Health Care Foundation Incorporated (no financial involvement).

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- Datasets are not publicly available because participants in the study did not give written consent for their data to be shared.
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If the above statements are not appropriate, the authors are asked to contact the JAFES Editorial Coordinator at jafes.editor@gmail.com.

Repositories for deposit of datasets may include institutional repositories or third-party repositories such as but not limited to:

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GENERAL GUIDELINES UPDATE

1. The manuscript should be encoded using Microsoft Word, double-spaced throughout with 1¼ cm (½ inch) paragraph indentation, with 3-cm margins (1¼ inch) all around on A4 size paper. The preferred font style and size is Times New Roman 12.
2. The manuscript should be arranged in sequence as follows: (1) Title Page, (2) Abstract, (3) Text, (4) References, (5) Tables, and (6) Figures & Illustrations.
3. References should pertain directly to the work being reported.
4. All the sheets of the manuscript should be labelled with the family name of the main author (all in capital letters) and page number (in Arabic Numerals) printed on the upper right corner.
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1. The title should be as concise as possible.
2. Only the full names of the authors directly affiliated with the work should be included (First name, Middle initial and Last name). There are 4 criteria for authorship (ICMJE recommendations):
   - 2.1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
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4. Name and location of no more than one (1) institutional affiliation per author may be included.
5. If the paper has been presented in a scientific forum or convention, a note should be provided indicating the name, location and date of its presentation.

Abstract

For original articles, the abstract should contain no more than 200 words with a structured format consisting of the objective/s, methodology, results and conclusion. For feature articles, case reports, interhospital grand rounds, and brief communications, the abstract should be from 50 to 75 words and need not be structured.

Keywords

At least 3 keywords but no more than 6, preferably using terms from the Medical Subject Headings (MeSH) list of Index Medicus, should be listed horizontally under the abstract for cross-indexing of the article.

Text

1. Generally, the text should be organized consecutively as follows: Introduction, Methodology, Results and Discussion, and Conclusion (IMRAD format).
2. All references, tables, figures and illustrations should be cited in the text, in numerical order.
3. All abbreviations should be spelled out once (the first time they are mentioned in the text) followed by the abbreviation enclosed in parentheses. The same abbreviation may then be used subsequently instead of the long names.
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6. Acknowledgments to individuals/groups of persons, or institution/s should be included at the end of the

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Use of Generative Artificial Intelligence (AI), such as ChatGPT, in drafting the manuscript or parts of the manuscript, shall be disclosed in the Methodology.

References

1. References in the text should be identified by Arabic Numerals in superscript on the same line as the preceding sentence.
2. References should be typed double-spaced on a separate sheet. They should be numbered consecutively in the order by which they are mentioned in the text. They should not be alphabetized.
3. All references should provide inclusive page numbers.
4. Journal abbreviations should conform to those used in PubMed. Include PMID, PMCID and DOI of the references.
5. A maximum of six authors per article can be cited; beyond that, name the first three and add "et al."
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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 November 18, 2005.

Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the US. JAMA. 2001;286(10): 1195-200. PMID: 11559264. https://doi.org/10.1001/jama.286.10.1195.

More than Six Authors

McGlynn EA, M.Asch S, Adams J, et al. The quality of health care delivered to adults in the United States. N Engl J Med. 2003;348(26):2635-45. PMID: 12826639. https://doi.org/10.1056/NEJMsa022615.

Jasul Jr. GV, Paz-Pacheco E, Jimeno CA, et al. AFES A.S.-O.N.E.: ASEAN Survey Of Needs in Endocrinology in the time of the COVID-19 pandemic. J AFES Fed Endocr Soc.2020;35(1):5-13. PMID:33790494. PMCID: PMC7992306. https://doi.org/10/15605/jafes.035.01.10.

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.

Tables

1. Cite all tables consecutively in the text and number them accordingly.
2. Create tables preferably using Microsoft Excel with one table per worksheet.
3. Tables should not be saved as image files.
4. The content of tables should include a table number (Arabic) and title in capital letters above the table, and explanatory notes and legends as well as definitions of abbreviations used below.
5. Font should be Arial Narrow size 8.
6. Each table must be self-explanatory, being a supplement rather than a duplicate of information in the text.
5. Up to a maximum of five (5) tables are allowed.

Figures and Graphs

1. Figures or graphs should be identified by Arabic Numeral/s with titles and explanations underneath.
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ARTICLE TYPES

Original Articles
The abstract should contain no more than 200 words with a structured format consisting of the objective/s, methodology, results and conclusion. A manuscript for original articles should not exceed 25 typewritten pages (including tables, figures, illustrations and references) or 6000 words.

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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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Images in Endocrinology
Authors may submit interesting, unique, rare, highly educational images from actual cases with an accompanying brief history and discussion. No abstract or keywords are necessary. The image should be at least 600 dpi. The write up should not exceed 500 words with a maximum of 10 references.

Checklist Guide for Submission of Manuscripts to JAFES	
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Keywords	☐ Provide 3-5 keywords (listed in MeSH)
Content	☐ Provide text/content in IMRAD format (Introduction, Methodology, Results and Discussion, Conclusion) ☐ Make sure all abbreviations are spelled out once (the first time they are mentioned in the text) followed by the abbreviation enclosed in parentheses; the same abbreviation may then be used subsequently ☐ Make sure all measurements and weights are in SI units ☐ If appropriate, provide information on institutional review board/ethics review committee approval ☐ Acknowledgments to individuals/groups of persons, or institution/s should be included at the end of the text just before the references; grants and subsidies from government or private institutions should also be acknowledged
References	☐ All references should be cited in the text, in numerical order. Use Arabic numerals ☐ Ensure all references follow the prescribed format
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Cover Letter

(Date)

To: **The Editor-in-Chief**
Journal of the ASEAN Federation of Endocrine Societies (JAFES)

Subject: **SUBMISSION OF MANUSCRIPT FOR PUBLICATION**

We intend to publish the manuscript/, entitled "_____," under the Section *[Original Article, Review Article, Feature Article, Case Report, Case Series, Interhospital Grand Rounds, Brief Communications, Letter-to-the-Editor, Special Announcements]* in the Journal of the ASEAN Federation of Endocrine Societies.

LIST OF AUTHORS

Complete Name	Position/ Designation	Institutional Affiliation	Role in writing the manuscript	Email address	ORCID iD

On behalf of all the authors, I shall act as the corresponding author with the journal from this point onward.

Attached herewith are the following: the completely accomplished **Author Form with author contribution disclosure** and **author publishing agreement**, in which all the authors certified authorship criteria was satisfactorily met and the specific contributions of the authors are listed and the author copyright is retained granting publishing and distribution rights to the JAFES; the **Author Declaration** that the work is original and is not under simultaneous consideration in other journals and the **ICMJE Disclosure forms** of ALL the authors (*where all conflicts of interest have been declared/there are no conflicts of interest*).

For original articles, we submit a scanned copy of our Ethics Review Approval/registration in trial registries (as appropriate) and the appropriate EQUATOR Network checklist used in writing the manuscript.

For case reports/series, patient consent forms have been secured for the publication of information.

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Furthermore, we respectfully suggest the following **reviewer**(s) for our manuscript.

Name and Salutation (e.g., Prof., Dr., etc)	Position/Designation	Institutional Affiliation and specialization	Email address

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Based on International Committee of Medical Journal Editors (ICMJE) Criteria for Authorship.

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