Volume 16, Number 3, November 2021

ISSN 1978-1059
EISSN 2407-0920

JURNAL GIZI DAN PANGAN

INDONESIAN JOURNAL OF NUTRITION AND FOOD

Jurnal Gizi dan Pangan

Volume 16, Number 3, November 2021

Articles

Published by:

Food and Nutrition Society of Indonesia

Department of Community Nutrition, Faculty of Human Ecology, IPB

Available online: http://journal.ipb.ac.id/index.php/jgizipangan **J. Gizi Pangan, March 2024, 19(1):1-10**
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2024.19.1.1-10*
ISSN 1978-1059 EISSN 2407-0920

Assessment of Adult Individuals' Fear of COVID-19, Healthy Living Behaviors, and Nutrition Knowledge Levels during the COVID-19 Pandemic Period

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ABSTRACT

This study determines the fear of COVID-19, healthy lifestyle behaviors, and nutrition knowledge levels of individuals during the COVID-19 pandemic. The study was conducted involving 509 people between the ages of 18 and 64 from December 2020 to April 2021. Data were collected online using a descriptive characteristics form, the COVID-19 Fear Scale, the Adult Nutrition Knowledge Level Scale, and the Healthy Lifestyle Behaviors Scale II. It was found from the study that women feared COVID-19 more than men ($p<0.05$). Individuals aged 35–44 scored higher in basic nutrition knowledge, while those aged 18–24 scored lower in food preference knowledge ($p<0.05$). It was also found that individuals who were between 55 and 64 years of age, married, highly educated, employed in the public sector, of good economic status, and ill scored higher on the Healthy Lifestyle Behaviors Scale II ($p<0.05$). A weak positive correlation was found between the score of the Fear of COVID-19 Scale and scores of both food preference knowledge and health responsibility sub-dimensions ($r=0.088$, $r=0.181$; $p<0.05$). A weak positive correlation was also found between the nutrition knowledge score as well as the different sub-dimensions and the total score of the Healthy Lifestyle Behaviors Scale II ($r=0.164$, $r=0.196$; $p<0.05$). It was observed that fear of COVID-19, nutrition knowledge level, and healthy lifestyle behaviors were influenced by various socio-demographic characteristics, and that there was a relationship between these three elements.

Keywords: fear of COVID-19, healthy lifestyle behaviors, nutrition knowledge level, pandemic

INTRODUCTION

The new coronavirus epidemic (COVID-19), which started in Wuhan, China, and spread worldwide, has led to a change in people's lifestyles (Nugroho *et al.* 2022; Alothman *et al.* 2021). Its high mortality rates, quarantine, social isolation, and worsening economy have caused widespread fear, stress, and anxiety (Pakpour & Griffiths 2020). Measures taken to limit the spread of the outbreak have affected people's physical and mental health, as well as their lifestyle behavior in many ways (Alothman *et al.* 2021). The increase in the time spent at home during the pandemic has caused negative changes in people’s lifestyle habits, such as unhealthy nutrition intake, low physical activity levels, and inefficient sleep (Pakpour & Griffiths 2020; Alothman *et al.* 2021).

It is believed that eating delicious food is a strategy to alleviate negative emotions such as anxiety, stress, and fear (Landaeta-Díaz *et al.* 2021). Stress and fear in quarantine may be linked to poor eating behaviors, such as eating even when not feeling hungry, consuming larger portions, and taking unhealthy foods (Pakpour & Griffiths 2020; Landaeta-Diaz *et al.* 2021). In a study by Cecchetto *et al.* (2021), it was found that individuals in COVID-19 quarantine more often displayed emotional and binge eating behaviors relating to stress, anxiety, and depression. In addition to changes in eating habits, restrictions in daily activities caused by isolation and quarantine have caused a great increase in energy imbalance (Landaeta-Diaz *et al.* 2021). Adoption of healthy lifestyle behaviors and an increase in nutrition knowledge levels can play an important role in the prevention of adverse health consequences arising at this time.

Nutrition knowledge can help individuals to form attitudes and behaviors towards healthy nutrition (Chen & Antonelli 2020). Some studies support the idea that having adequate nutrition knowledge can positively affect nutrition habits (Spronk *et al.* 2014; Barbosa *et al.* 2016). In

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(Received 08-08-2023; Revised 24-11-2023; Accepted 11-12-2023; Published 30-03-2024)

one systematic review involving adults, higher levels of nutritional knowledge were generally associated with healthier food choices and eating habits (Barbosa *et al.* 2016).

During the COVID-19 pandemic, it is necessary to identify the current situation so that adults can both protect and maintain their health and develop mechanisms to cope with negative feelings (Cecchetto *et al.* 2021). Thus, negative eating habits that may be caused by mental distress can be prevented, and lifestyle can be improved in the fight against future pandemics. The aim of this study is to investigate fear of COVID-19, healthy lifestyle behaviors, and nutrition knowledge during the pandemic period in people aged 18–64 years

METHODS

Design, location, and time

Data collection was performed online between December 2020 and April 2021 through a survey form prepared and created by the researcher based on literature using Google Forms. All participants had read the declaration of consent and expressly agreed to participate before starting the survey. The study protocol was approved by the Ethics Committee of Ankara Yıldırım Beyazıt University (09.11.2020-53) and by the Scientific Research Evaluation Commission of the Health Services General Directorate of the Turkish Ministry of Health (2020-10-18T13_55_56).

Sampling

This study was conducted with 509 individuals aged between 18 and 64 years. The survey form was distributed via social media platforms. The eligibility criteria included being aged 18–64 years, proficiency in Turkish, ownership of a smart phone or computer. Taking into account the Turkey population size, and employing a confidence level of 95% and a sampling error of 5%, the minimum sample size necessary for the study was calculated to be 377.

Data collection

Descriptive characteristics form. This form was used to collect descriptive information on the individuals involved in this study. Their Body Mass Index (BMI) was calculated by the researcher according to their own statements regarding their body weight (kg) and height (cm).

COVID-19 Fear Scale (CFS). This scale was developed in 2020 by Ahorsu *et al.* to determine the level of fear of COVID-19 (Ahorsu *et al.* 2020), and Satıcı *et al.* conducted a Turkish validity and reliability study on it in 2020. The scale uses a five-point Likert scale format and consists of seven items. A high score on the scale indicates a high level of COVID-19 fear (Satici *et al.* 2021).

Adult Nutrition Knowledge Level Scale (ANKLS). This scale was developed by Batmaz and Güneş (2018) to determine individuals' nutrition knowledge levels and later subjected to validity and reliability testing. The scale is in a five-point Likert scale form and has two sections, Basic Nutrition and Nutrition Preference. There are 20 items in the Basic Nutrition section and 12 items in the Nutrition Preference section.

Healthy Lifestyle Behaviors Scale II (HLBS-II). This scale, originally developed in 1987, was updated in 1996 by Walker *et al.* to assess people's health behavior (Walker *et al.* 1987; Walker & Hill-Polerecky 1996). The validation and reliability of the Turkish version was tested by Bahar *et al.* (2008). The scale has a four-page Likert-type format and comprises 52 items in six sub-dimensions: mental development, interpersonal relationships, nutrition, physical activity, health responsibility, and stress management. The possible score range is from 52 to 208.

Data analysis

Data analysis was performed using SPSS (IBM SPSS Statistics, Version 24). The qualitative variable data are summarized as number (S) and percentage (%), while the quantitative variable data are summarized as mean and standard deviation ($\bar{X}$±SD). For normally distributed data, independent samples t-test and ANOVA were employed. For three or more groups, the Tamhane test was performed in a two-way comparison of variables. For non-normally distributed data, the Mann-Whitney U test and the Kruskal-Wallis H test were used. The Bonferroni correction was performed for the two-way comparison of variables, for which a significant difference emerged for groups of three or more. For categorical variables, the Pearson chi-square test was used to compare between groups, and the Spearman correlation test was used to determine the correlation between quantitative variables. Data analysis was assessed with a 95% confidence interval and a 5% significance level, and statistical significance was accepted as $p<0.05$.

RESULTS AND DISCUSSION

Table 1 presents the socio-demographic characteristics and state of having a disease of the individuals. Of the participants, 58.7% were female with an average age of 30.83±10.40 years. Notably, 54.6% had a BMI within the normal range, but 39.1% mentioned gaining weight during the pandemic.

Table 2 provides the scores of the individuals on the CFS, ANKLS, and HLBS-II. The score obtained on the CFS was 16.97±6.17. The scores obtained from the basic nutrition section and nutrition preference section of the ANKLS were 54.31±7.69 and 37.05±6.29, respectively. The individuals' HLBS-II score was 121.11±19.24. The scores obtained on the sub-dimensions of this scale, health responsibility, nutrition, and physical activity, were 18.51±4.49, 19.99±3.86, and 15.68±5.17, respectively.

Table 3 shows the distribution of the scores obtained from the CFS, ANKLS, and HLBS-II according to the individuals' descriptive characteristics. Women had a significantly higher CFS score than men ($p<0.05$). The basic nutrition knowledge scores of those in the 35–44 years age range were higher, and the nutrition preference knowledge scores of those in the 18–24 years age range were seen to be lower ($p<0.05$). The total HLBS-II score of the individuals in the 55–64 years age range was found to be higher ($p<0.05$). The total points of individuals who were married on both the ANKLS and HLBS-II were significantly higher than those of unmarried individuals ($p<0.05$). It was observed that the total scores of both the ANKLS and HLBS-II for individuals with postgraduate education were notably higher ($p<0.05$). Additionally, those with a reported illness had significantly higher scores than those without ($p<0.05$).

Table 4 examines the correlation between the individuals' CFS, ANKLS, and HLBS-II scores and sub-dimensions. A weak positive significant correlation was observed between the CFS score and nutrition preference ($r=0.088$, $p=0.048$) and the score of only the sub-dimension of health responsibility of the HLBS-II ($r=0.181$, $p<0.001$). Also, weak positive significant correlations were found between the nutrition preference score and health responsibility ($r=0.189$, $p<0.001$), nutrition ($r=0.136$, $p=0.002$), spiritual development ($r=0.151$, $p=0.001$), interpersonal relations ($r=0.139$, $p=0.002$), stress management ($r=0.187$, $p<0.001$), and the HLBS-II total score ($r=0.196$, $p<0.001$).

The COVID-19 pandemic has been unpredictable and has affected the whole world. Its rapid spread, high death rate, uncertain course, and economic disruption have caused fear in the general population (Pakpour & Griffiths 2020). In this study, individuals' CFS score was found to be 16.97±6.17. In March and April 2020 when the outbreak first began, another study conducted in Turkey found a CFS score of individuals of 21.47±6.28 (Korukcu *et al.* 2021). In a similar study conducted in Saudi Arabia with individuals over the age of 18, the CFS score found was 16.28±5.49 (Alothman *et al.* 2021). It is noticeable from the studies conducted that COVID-19 fear levels have changed over time. At the beginning of the normalization process, the progressive fall in death rates and increase in vaccination rates might have reduced the fear levels.

In this study, fear of COVID-19 was higher in women than in men ($p<0.05$). It has been similarly reported in many other studies in the literature that females have greater fear of COVID-19 (Pakpour & Griffiths 2020; Taspinar *et al.* 2021). In a study in Bangladesh in which 8,550 people took part, females reported significantly higher levels of fear of COVID-19 than males (Sakib *et al.* 2022). Females reporting greater fear of COVID-19 may be explained by physiological and psychological differences between the genders. Estrogen levels and hormonal fluctuations can modulate fear responses in women (Maeng & Milad 2015).

In the present study, the basic nutrition information scores of individuals in the 35–44 years age group were higher than those of other age groups, and the nutrition preference information scores of those in the 18–24 years age group were lower than those of other age groups ($p<0.05$). In addition, nutrition preference information scores increased with increasing age. In a study by Batmaz and Güneş (2018), it was similarly found that scores on nutrition preference increased with age. In a study by Hendrie *et al.* (2008), it was found that nutrition

Table 1. Distribution of individuals' general descriptive characteristics

General characteristics	Individuals (n=509)	
	S	%
Gender		
Female	299	58.7
Male	210	41.3
Age range (years)		
18–24	159	31.2
25–34	207	40.7
35–44	77	15.2
45–54	48	9.4
55–64	18	3.5
Age (years) ($\bar{X}$±SD)	30.83±10.40	
Marital status		
Married	191	37.5
Single	318	62.5
Education		
Middle school	27	5.3
High school	102	20.0
University degree	288	56.6
Postgraduate	92	18.1
Employment		
Not working	223	43.8
Public sector	148	29.1
Private sector	138	27.1
Chronic illness		
Yes	104	20.4
No	405	79.6
Body mass index range		
Underweight	28	5.5
Normal	278	54.6
Moderately overweight	152	29.9
Obese	51	10.0
Weight change		
Increase	199	39.1
Decrease	110	21.6
No change	200	39.3

general information increased with age, and that individuals over the age of 35 had a higher level of nutrition knowledge than those who were younger. The low nutrition information scores of individuals in the 18–24 years age range in the present study might be because these individuals had inadequate education on nutrition. In addition, the presence of chronic illnesses which developed with age and the elevation in sociocultural and education levels might have caused the increase in nutrition knowledge levels with increasing age (Tam *et al.* 2021; Akkartal & Gezer 2020).

It is noticeable that although studies conducted before the pandemic reached varying conclusions, it was generally found that females' nutrition knowledge levels were higher (Hendrie *et al.* 2008; Labban 2015). This finding regarding the gender factor may be due to the fact that women attach more importance to healthy nutrition and weight control (Spronk *et al.* 2014; Lee *et al.* 2019). In the present study, no significant difference was found between gender and the nutrition knowledge score (p>0.05). Unlike the conclusions of other studies, the conclusions of this study can be explained by the data collected during the pandemic. It was assumed that the pandemic period was an important factor in the increase in the levels of knowledge about healthy eating to strengthen immunity irrespective of gender.

The basic nutrition and nutrition preference scores of those who were married were higher than the scores of unmarried individuals (p<0.05). Similarly, it was found in a study by Hendrie *et al.* (2008) that individuals who were married, divorced, or living together with another individual or other individuals had higher levels of nutrition knowledge than those who were unmarried. Marriage potentially offers both economic and social advantages. This increased economic prosperity can improve health outcomes by increasing access to healthcare or reducing stress. Additionally, the partner can play an important role in monitoring and encouraging healthy behaviors (such as good eating habits and regular exercise) (Wood *et al.* 2009).

Existing studies in the literature have shown that education status is an important factor influencing nutrition knowledge levels (Koch *et al.* 2021; Tam *et al.* 2021; Akkartal & Gezer 2020). In the present study, nutrition levels generally rose as education levels rose. Tam *et*

Table 2. Distribution of CFS, ANKLS and HLBS-II scores

Scales	Mean	SD	Median	Lower	Upper
COVID-19 fear scale adult nutrition knowledge level scale	16.97	6.17	16.0	7.0	35.0
Basic nutrition	54.31	7.69	54.0	31.0	76.0
Nutrition preference	37.05	6.29	37.0	8.0	48.0
Healthy lifestyle behaviors scale					
Health responsibility	18.51	4.49	18.0	9.0	30.0
Physical activity	15.68	5.17	15.0	8.0	32.0
Nutrition	19.99	3.86	20.0	10.0	31.0
Spiritual development	24.98	4.82	25.0	10.0	36.0
Interpersonal relations	23.46	4.19	23.0	12.0	35.0
Stress management	18.49	3.91	18.0	9.0	32.0
Total HLBS II	121.11	19.24	120.0	69.0	178.0

SD: Standard Deviation; CFS: COVID-19 Fear Scale; ANKLS: Adult Nutrition Knowledge Level Scale
HLBS-II: Healthy Lifestyle Behaviors Scale II

al. (2021), who assessed the nutrition knowledge of Australian sportsmen, found that sportsmen with a university education had higher nutrition knowledge scores. These findings have shown that education increases individuals' awareness levels, which represents an important factor in achieving better levels of nutrition knowledge.

In a study by Taş (2021), no statistically significant difference was found when individuals' chronic illness status and nutrition knowledge levels were compared. In the present study, the basic nutrition scores of those with a chronic disease diagnosed by a doctor were higher than those of individuals without an illness ($p<0.05$). The different results seen in the literature on this topic might have derived from whether or not the sick individuals had education, or if they had education, whether it was adequate. Also, the result might have varied according to whether those who were ill had an illness related to nutrition.

The HLBS-II was used to determine the healthy lifestyle behaviors of the individuals included in the study, and their total mean score was found to be 121.11±19.24. Few studies have been in use of this scale with the general population involved (Liu *et al.* 2021; Akgün 2021; Zhou *et al.* 2022). Similar to the present study, Akgün (2021) conducted a study to determine the healthy lifestyle behaviors of individuals above the age of 18 during the pandemic, and the total HLBS-II score was 123.49±18.47. Considering that the score that can be obtained from the HLBS-II may fall in the range of 52–208, it is predicted that adults will need to enhance their health improvement behaviors.

Based on the scores on the sub-dimensions of the HLBS-II, individuals scored the lowest, 15.68±5.17, on physical activity (min score: 8, max score: 32). The fact that existing literature has reported the lowest score on this sub-dimension shows that adults do not incorporate physical activity into their lifestyle (Zhou *et al.* 2022; Alzahrani *et al.* 2019). The reason for this may be that adults do not attach enough importance to physical activity, and that recently developed technology has changed lifestyles. In addition, people spend more time at home and adopt a sedentary lifestyle because of the measures taken during the COVID-19 pandemic, and this may have been an important factor in the physical activity sub-dimension having the lowest score.

In one study, nurses were divided into age groups of 21–30 years, 31–40 years, and 41 years and over, and it was found that those aged 41 and over had a significantly higher HLBS-II total score. The increase in the HLBS-II score average with age is attributed to individuals having more knowledge and experience in the field of health, leading a more regular lifestyle, and giving more importance to their health due to health problems that arise with advancing age (Altay *et al.* 2015). In the present study, the total HLBS-II score of those in the 55–64 years age group was higher.

Table 3. Comparison of individuals' CFS, ANKLS and HLBS-II total mean scores according to various descriptive characteristics

General characteristics	COVID-19 Fear scale (CFS)		Adult nutrition knowledge level scale (ANKLS) Basic nutrition		Adult nutrition knowledge level scale (ANKLS) Nutrition preference		Healthy lifestyle behaviors scale II (HLBS- II) total	
	Mean±SD	Test statistic *p*	Mean±SD	Test statistic *p*	Mean±SD	Test statistic *p*	Mean±SD	Test statistic *p*
Gender								
Female	18.21±6.22	Z=-5.350*	54.61±7.87	Z=-0.488*	37.52±6.20	Z=-1.906*	120.08±19.19	t=-1.440***
Male	15.25±5.66	**p<0.001**	53.87±7.43	p=0.626	36.37±6.37	p=0.057	122.57±19.25	p=0.150
Age range								
18–24(1)	16.55±6.28	χ^2=6.306**	52.78±7.81	χ2=19.753**	35.03±6.50	χ^2=24.571**	115.85±18.14	χ^2=27.087**
25–34(2)	17.05±5.98	p=0.177	54.14±7.74	**p=0.001**	37.42±6.50	**p<0.001**	122.14±18.59	**p<0.001**
35–44(3)	17.16±5.90		56.96±6.76	**[3–1,2]**	38.57±4.98	**[1–2,3,4]**	121.83±18.26	[1–2,4,5]
45–54(4)	16.58±6.25		55.89±7.39		38.87±5.27		128.29±22.21	
55–64(5)	20.44±7.57		54.22±7.64		39.33±4.95		133.22±19.79	
Marital status								
Married	16.92±6.37	Z=-0.436*	55.61±7.25	Z=-2.94*	38.86±5.59	Z=-4.939*	124.72±19.04	Z=-3.570*
Single	17.03±6.05	p=0.663	53.53±7.85	**p=0.003**	35.96±6.44	**p<0.001**	118.94±19.06	**p=0.001**
Education								
Middle school	17.37±6.79	χ^2=2.359**	49.26±7.35	χ^2=31.348**	36.74±6.99	χ^2=22.996**	116.07±19.56	χ2=14.251**
Highschool	16.65±6.68	p=0.501	52.53±7.33	**p<0.001**	34.44±6.70	**p<0.001**	117.40±19.88	**p=0.003**
University	16.86±5.98		54.47±7.19	**[4–1,2,3]**	37.48±5.76	**[2–3,4]**	121.09±18.61	**[4–1,2]**
Postgraduate	17.64±6.01		57.28±8.49		38.68±6.45		126.75±19.27	
Employment								
Not working	17.02±6.38	χ^2=0.122**	53.16±7.67	χ^2=15.799**	36.17±6.39	χ^2=14.499**	118.18±19.02	χ^2=10.567**
Public	16.84±5.74	p=0.941	56.14±7.79	**p<0.001**	38.58±6.01	**p=0.001**	125.30±20.86	**p=0.005**
Private	17.09±6.29		54.22±7.29	**[1–2]**	36.84±6.16	**[1–3]**	121.35±16.93	**[1–2]**
Illness								
Yes	18.06±6.36	Z=-1.949*	56.16±7.68	Z=-2.082*	38.26±5.56	Z=-1.835*	124.43±17.36	Z=-2.296*
No	16.71±6.09	p=0.051	53.84±7.63	**p=0.037**	36.74±6.43	p=0.067	120.26±19.63	**p=0.022**
Body mass index								
Underweight	19.00±6.48	χ^2=2.674**	53.43±7.14	χ^2=1.934**	36.46±5.90	χ^2=6.189**	113.21±19.04	F=2.125****
Normal	16.88±5.99	p=0.445	54.01±7.83	p=0.586	36.83±6.42	p=0.103	120.27±18.38	p=0.096
Moderately overweight	16.91±6.38		54.57±6.84		36.92±6.36		125.52±20.15	
Obese	16.67±6.29		55.69±9.46		39.00±5.31		122.86±20.22	

Mean±SD: Mean±Standard Deviation; *: Mann-Whitney U test; **: Kruskall-Wallis H test; ***: Independent Sample-t Test; ****: ANOVA test

p<⁰.⁰⁵ was accepted as statistically significant

Those aged 18–24 might have scored lower because in this age range individuals are still pursuing studies, or because of economic factors, irregular lifestyles, and insufficient knowledge and experience.

Although the pandemic has caused adverse changes in individuals' lifestyle behaviors, such as decreased physical activity, insufficient sleep, or poor psychological health (Alothman *et al.* 2021), it has also improved health-related knowledge and attitude (Aksoy *et al.* 2021). In one study, it was figured out that COVID-19 fear had a positive effect on health knowledge and attitude, and that attitude was positively correlated with

Table 4. Correlation between individuals' scale scores

Scales		CFS	ANKLS-Basic nutrition	ANKLS-Nutrition preference	HLBS-Health responsibility	HLBS-Physical activity	HLBS-Nutrition	HLBS-Spiritual development	HLBS-Interpersonal relations	HLBS-Stress management
ANKLS-Basic nutrition	r	-0.017								
	p	0.707								
ANKLS-Nutrition preference	r	**0.088**	**0.617**							
	p	**0.048**	**<0.001**							
HLBS-Health responsibility	r	**0.181**	**0.120**	**0.189**						
	p	**<0.001**	**0.007**	**<0.001**						
HLBS-Physical activity	r	-0.058	0.061	0.032	**0.383**					
	p	0.194	0.166	0.468	**<0.001**					
HLBS-Nutrition	r	0.051	**0.205**	**0.136**	**0.422**	**0.354**				
	p	0.255	**<0.001**	**0.002**	**<0.001**	**<0.001**				
HLBS-spiritual development	r	-0.050	**0.145**	**0.151**	**0.437**	**0.335**	**0.322**			
	p	0.261	**0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**			
HLBS-Interpersonal relations	r	0.048	0.036	**0.139**	**0.497**	**0.279**	**0.298**	**0.590**		
	p	0.279	0.418	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		
HLBS-Stress management	r	0.020	**0.112**	**0.187**	**0.427**	**0.454**	**0.381**	**0.577**	**0.502**	
	p	0.651	**0.011**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	
Total HLBS II	r	0.027	**0.164**	**0.196**	**0.722**	**0.650**	**0.612**	**0.772**	**0.733**	**0.755**
	p	0.540	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

CFS: COVID-19 Fear Scale; ANKLS: Adult Nutrition Knowledge Level Scale; HLBS: Healthy Lifestyle Behaviors Scale

healthy eating (Aksoy *et al.* 2021). In the present study also, it was found that as COVID-19 fear levels increased, nutrition preference knowledge levels also increased. The reason for the positive correlation between COVID-19 fear and nutrition preference knowledge level might be that people wanted to have a strong immune system and preferred healthy food.

It is predicted that in times of pandemic, the fear which arises may have a negative effect on lifestyle behaviors by increasing levels of stress and worry (Shultz *et al.* 2016). However, it was seen in the present study that the fear which occurred during the pandemic could also motivate positive lifestyle behaviors as in the health responsibility sub-dimension (Harper *et al.* 2021). It was seen in this study that as people's COVID-19 fear levels increased, there was a positive development in their health responsibility behaviors. Existing literature has shown that there is a mixed correlation between fear and health-related behaviors (Pakpour & Griffiths 2020; Farías 2020; Demirtaş-Madran 2021). In a study by Harper *et al.* (2021), it was found that fear of COVID-19 increased the perception of risk and led to positive changes in health protection behaviors. Many theories in the literature have shown that fear-based messages lead to positive changes (Demirtaş-Madran 2021). Messages, called "fear appeal" in health communication, are perceived as threatening and stimulating fear. The effectiveness of such messages stems from the tendency to believe in the persuasive power of inducing fear for positive or negative behavior (Pakpour & Griffiths 2020).

In this study, it was found that as people's basic nutrition knowledge levels increased, their health responsibility, nutrition status, spiritual development, stress management, and total HLBS-II score also increased. Also, it was figured out that as the nutrition preference knowledge scores of those participating in the study increased, their general healthy lifestyle behaviors also increased. The conclusions of the present study accord with some studies, which show a weak positive correlation between nutrition knowledge and diet quality (Spronk *et al.* 2014; Koch *et al.* 2021). In a study by Zaborowicz *et al.* (2016), health-improving behaviors such as not adding sugar to drinks and not putting salt on food were found to be commoner in individuals with high levels of nutrition knowledge. Some studies have shown that having a good level of nutrition knowledge is not always correlated with healthier nutrition habits (Aktaç *et al.* 2018; Suliga *et al.* 2020). In a study conducted on Polish, German, and Slovak students, it was found that Polish students had the highest knowledge levels on the topic of food and nutrition, but this was not reflected in their diet (Suliga *et al.* 2020). These findings show that an increase in nutrition knowledge may not always be reflected in behavior. This is because eating habits are influenced by many factors, including personal factors (biological characteristics and physiological needs, habits and experiences, and psychological components), cognitive factors (knowledge and skills, attitudes, tastes and preferences, expected outcomes, and personal identity), and sociocultural factors (economic variables, culture, and political elements) (Chen & Antonelli 2020). Also, even though significant correlations were observed in the expected direction between nutrition knowledge level and healthy lifestyle behaviors in this study, this correlation was at a weak level. Thus, it is seen that it would not be possible for the rise in nutrition knowledge alone to lead to large changes in nutrition and other health lifestyle behaviors.

This research has some limitations. As the research data were collected online, the participants were limited to those who could use information technologies such as computers or mobile phones. Research data were collected in a self-reported manner and thus became subjective. It was assumed that the validity and reliability of data that could be measured by the researcher, especially body weight and height, were higher. These limitations were a consequence from the threats and restrictions brought by the COVID-19 pandemic.

CONCLUSION

This study found that fear of COVID-19, nutritional knowledge, and healthy lifestyle behaviors were influenced by various socio-demographic characteristics and that there was a relationship between these three items.

The expected positive but weak correlation between nutrition knowledge level and lifestyle behaviors showed that an increase in knowledge level by itself was not enough to cause behavioral changes. Therefore, effective education should be planned, so that individuals may adopt lifestyle behaviors such as healthy eating and physical activity and put knowledge into practice. This education should be arranged so as to meet individuals' needs by taking into account individual, social, and environmental factors.

ACKNOWLEDGEMENT

The authors would like to acknowledge all the participants.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2024, 19(1):11-20***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2023.19.1.11-20*
ISSN 1978-1059 EISSN 2407-0920

Nutritional Assessment among Type 2 Diabetes Mellitus Patient in Southeast Asian Countries: A Scoping Review

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ABSTRACT

This scoping review aims to determine the available nutritional assessments for people with Type 2 Diabetes Mellitus (T2DM) in Southeast Asian countries. The methodology used for this research was based on the PRISMA-ScR standards. An extensive electronic search was carried out for papers published between 2012 and 2022 that pertained to studies conducted in Southeast Asian countries and were written in English. The eligibility criteria for this review were T2DM patients aged 20 years and older. The search was carried out using PubMed, Science Direct, Scopus, and Google Scholar databases. Hence, out of 5,445, fourteen articles met the eligibility requirements of the analysis. According to the findings, twelve studies used anthropometry measurements and biochemical tests, followed by eight studies using clinical assessments and four studies using dietary assessments. The research utilized various nutritional assessment methods such as weight, height, Body Mass Index (BMI), waist and hip circumference, body fat percentage, Fasting Blood Glucose (FBG), Glycated Hemoglobin (HbA1c), lipid profiles, Blood Pressure (BP), 3-day and 24-hour dietary recall. This review examined how the available nutritional assessments for T2DM are frequently carried out in Southeast Asian countries. The review discovered that weight, height, BMI, waist and hip circumference, FBG, HbA1c, BP, and 3-day dietary recall are the most commonly reported nutritional assessment methods.

Keywords: nutritional assessments, Southeast Asian countries, type 2 diabetes mellitus

INTRODUCTION

One of the first diseases and metabolic conditions known to man is diabetes mellitus (DM), which involves excessively elevated blood glucose levels. Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) are the two main subtypes of Diabetes Mellitus (DM). T1DM and T2DM are primarily brought on by faulty insulin production and action, respectively (Sapra & Bhandari 2021). T1DM presents in children or adolescents, while T2DM is thought to affect middle-aged and older adults who have prolonged hyperglycemia due to poor lifestyle and dietary choices (Sapra & Bhandari 2021).

Type 2 diabetes is becoming more and more commonplace globally, and there are no indications that this trend will slow down. The International Diabetes Federation (IDF) states that those in their 20s to 79s are particularly at risk for developing diabetes in South Asia (SA) and Southeast Asia (SEA). Diabetes roughly affects over 88 million adults in the IDF South-East Asia Region between the ages of 20 and 79. This represents almost 9% of the regional population in this age group (International Diabetes Federation (IDF) 2021). According to IDF predictions, by 2045, the SEA Region would see a 68% increase in the number of diabetics, reaching 152 million people and a 30% increase in the prevalence of diabetes, to 11.3%. Additionally, with 51.2% of cases remaining undiagnosed, according to the IDF (2021), the SEA Region has the third-highest rate of diabetes worldwide. As such, diabetes

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(Received 04-10-2023; Revised 18-11-2023; Accepted 03-01-2024; Published 30-03-2024)

continues to rank among the leading causes of major health problems.

A study by Selph *et al.* (2015) discovered that Glycated Hemoglobin (HbA1c) tests and fasting glucose levels are crucial for the early diagnosis of type 2 diabetes. The American Diabetes Association (ADA) states that a diagnosis of diabetes may come from any of the following: a HbA1c reading of at least 6.5%; 7.0 mmol/L or greater fasting plasma glucose (no caloric intake for at least 8 hours); Nonetheless, the ADA advises screening everyone 45 years of age and older, regardless of risk, while the United States Preventive Services Task Force suggests screening overweight people 40 to 70 years of age (Selph *et al.* 2015).

The nutritional assessment is a complex procedure that collects, organizes, and integrates data from the clinical, laboratory, and paraclinical domains in which enables healthcare professionals to systematically evaluate a patient's overall nutritional status, diagnose malnutrition, identify underlying pathologies that cause malnutrition, and prepare necessary interventions; thus, nutritional status has an impact on clinical outcomes (Kesari & Noel 2022). When performing nutritional assessments, it is imperative to remember that there is no one test that is ideal for determining nutritional status. To assess the population's nutritional status, a systematic data collection process is required, and all available data should be analysed. An extensive clinical examination (history and physical examination), anthropometric measurements, diagnostic testing, and dietary assessments should all be part of a comprehensive nutritional assessment, according to the American Society for Parenteral and Enteral Nutrition (ASPEN) (Kesari & Noel 2023).

The diagnosis methods, intervention thresholds, management goals, and instruments utilised to attain them—collectively, the "four T's"—must be reasonable, practical, and consistent with dietary and lifestyle choices. Because of this, many Southeast Asian countries have established their own national guidelines, such as Clinical Practice Guidelines (CPGs), that are appropriate for their particular circumstances (Kalra *et al.* 2017). However, as far as we are aware, no review article has ever been published that addresses nutritional assessments for T2DM patients in Southeast Asian countries. Health practitioners need to be better informed about the current methods used in nutritional assessments among T2DM patients if they want to ensure and improve the management of the condition. This scoping review sought to examine the most recent studies on the available nutritional assessment of T2DM patients in Southeast Asian countries.

METHODS

The associated publications were reviewed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) criteria as a guide (Tricco *et al.* 2018). The original research papers that were published between 2012 and 2022 were the subject of the current scoping review, which included the following steps: (i) identifying the research question; (ii) identifying search strategies; (iii) study selection; (iv) data charting; and (v) collating, summarizing, and reporting the results (Tricco *et al.* 2018).

Identifying the research question

This scoping review's objective was to determine the available nutritional assessments (anthropometry, biochemical, clinical and dietary) of T2DM patients in Southeast Asian countries. The research question was; What are the available nutritional assessments conducted among T2DM patients in Southeast Asian countries?

Identifying search strategies

The "Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR)" approach served as the foundation for the design of this scoping review (Tricco *et al.* 2018). Four separate electronic databases—PubMed, Science Direct, Scopus, and Google Scholar—were accessed in order to do a comprehensive search utilising dependable resources to locate the original English-language research papers published between 2012 and 2022.

There are three stages to the document this selection procedure. In the first phase, keywords for the search were determined. Nutritional, dietary, nutrition, assessment, evaluation, status, type 2 diabetes mellitus, patients, adults, and Southeast Asian countries such as Brunei, Myanmar, Cambodia, Timor-Leste, Indonesia, Laos, Malaysia, the Philippines, Singapore, Thailand, and Vietnam were among the terminology utilized by the database. Millions of combinations of important search terms were used by the search techniques, including: (i) (nutritional OR dietary OR nutrition) AND (assessment OR evaluation OR status) AND; (ii) (type 2 diabetes mellitus) AND (patients OR adults) AND; (iii) (Southeast Asian countries OR Brunei OR Myanmar OR Cambodia OR Timor-Leste OR Indonesia OR Laos OR Malaysia OR Philippines OR Singapore OR Thailand OR Vietnam).

Study selection

For Phase 2, the screening phase, the selection criteria were established by the researchers whom were working in pairs. The data gathered by the researchers was compared, and any differences between the reviewers were discussed, to guarantee consistency in study selection. The nutritional assessment for T2DM patients served as the primary selection criterion for this scoping review to identify relevant articles. Articles that satisfied the inclusion criteria were deemed admissible for this review. First, to ensure the acquisition of relevant, recent data, the inclusion criteria for this review were English-language original research papers published between 2012 and 2022. From then on, T2DM patients became the study's inclusion criteria for participants and at least 20 years old, whereas the exclusion criteria were children and teenagers (aged under 20 years old) and people with T1DM and GDM. Finally, this review only included the Southeast Asian countries; Brunei, Myanmar, Cambodia, Timor-Leste, Indonesia, Laos, Malaysia, the Philippines, Singapore, Thailand, and Vietnam (SarDesai 2018).

During Phase 3, the articles that did not fit the requirements for inclusion criteria were removed through the eligibility process whereby, papers that were systematic, literature-based, or other review papers were excluded. To determine if the content was appropriate for the review, a thorough screening process was used to the titles and abstracts of the chosen papers. Using the inclusion and exclusion criteria, the researchers (working in pairs) excluded irrelevant papers that did not address the research topics. Excluded were abstracts and titles that had no bearing on the study's objectives.

Data charting

Using the eligible titles and abstracts as a guide, the researchers downloaded the soft copies of the full articles from the databases. The articles were reviewed to find out if the whole article addressed the objective and research question. Version 2.80.1 of the Mendeley software was used for data administration. A Microsoft Excel spreadsheet was then used to record the information that had been taken out of the complete articles. One researcher independently performed the data graphing, and another (working in pairs) confirmed it. A table comprising the following details was created by extracting and charting the general and specific data from the chosen studies: author(s), year of publication, country, sample characteristics (sample size, study design, population, and age), and nutritional assessment (anthropometry, biochemical, clinical, and dietary).

Collating, summarising, and reporting the results

Table 1 summarizes and tabulates the extracted data results. Some limitations of the studies were highlighted to provide useful recommendations for future references on nutritional assessment in T2DM patients.

RESULTS AND DISCUSSION

Study selection

The total number of titles and abstracts found in the databases search were 5,445; in which PubMed (221), Science Direct (1,819), Scopus (57), and Google Scholar (3,348). The duplicate articles were managed using the Mendeley software through recording, monitoring, sorting, and checking the studies, resulting in a total of 255 sets of duplicates being eliminated from the list. After eliminating duplicates, a total of 5,190 abstracts were included in the initial screening phase. However, 5,146 abstracts were discarded due to various reasons. These exclusions were based on criteria such as the removal of articles published in 2011 and earlier (410), non-English articles (213), titles and abstracts unrelated to the review (2,770), and non-Southeast Asian countries (370). Among the 5,146 abstracts, the publications that were published as reviews which were part of systematic, literature, and scoping reviews were also excluded (1,383). From the 5,146 abstracts, only 44 potentially relevant publications were chosen for eligibility checking by reading their full text. After the full texts of these 44 papers were evaluated for eligibility, fourteen publications were included in the final data collection. A flowchart of the study selection procedure is shown in Figure 1.

Study characteristics

The review papers' study characteristics were summarized in Table 1. Location, study design, sample size, population, age, and nutritional assessment (anthropometry, biochemical, clinical, and dietary) were the categories used to group the abstracted data. Seven studies from Indonesia, three from Malaysia, three from Singapore, and one from Thailand were covered in the papers. Most research designs were cross-sectional and quasi-experimental, followed by prospective observational, retrospective, cohort, clinical trial, and randomized controlled trial studies. T2DM patients made up the entire study group, and they ranged in age from young adults in their early 20s to elderly adults.

Nutritional assessments of T2DM patients

An overview of the nutritional assessments used in the chosen research is shown in Table 1. Among the fourteen reviewed articles, twelve used anthropometry measurement, twelve used biochemical tests, eight used clinical, and four used dietary intakes as their parameters (Table 1). Most of the studies measured the individuals' height, weight, and BMI for anthropometry. However, several studies also measured body fat percentage, hip circumference, and waist circumference. Regarding biochemical testing, the studies assessed revealed (twelve articles) that the tests typically performed among the subjects were HbA1c, Fasting Blood Glucose (FBG), and lipid profile tests for determining the individuals' glycaemic control and cholesterol levels.

In eight of fourteen reviewed articles pertaining to clinical assessments, the blood pressure of the subjects was measured during the assessment. Four articles revealed that the subjects' dietary intake was assessed throughout the assessment process using the 3-day dietary recall (2 weekdays and 1 weekend) and 24-hour dietary recall.

Anthropometry

The anthropometry measurements were reported in twelve of the fourteen analysed

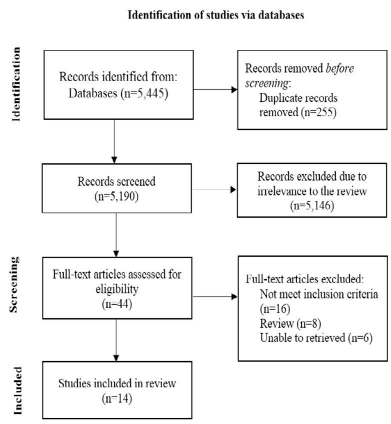

Figure 1. PRISMA-ScR method flowchart

studies, six of which were from Indonesia, two from Malaysia, three from Singapore, and one from Thailand (Table 1). The assessments took into account the following factors: height, weight, Body Mass Index (BMI), hip and waist measurements, and body fat percentage.

According to research conducted in Malaysia, 62% of the participants were either obese or overweight, and most of them displayed indications of abdominal obesity, and women had a substantially higher mean BMI than males ($p<0.05$) (Firouzi *et al.* 2015). Another study from Indonesia (Andriani & Maria 2022) reported the majority of patients had poor nutritional status (56.6%), whereas a study from Thailand (Thewjitcharoen *et al.* 2018b) reported the majority (80.2%) met the WHO-recommended BMI cut-offs for Asians, indicating they were overweight or obese. Besides that, according to Thewjitcharoen *et al.* (2018b) and Andriani & Maria (2022), the following were the BMI categories: (a) underweight: 18.5 kg/m^2; (b) normal: 18.5–22.9 kg/m^2; (c) pre-obese: 23–27.4 kg/m^2; and (d) obese: 27.5 kg/m^2. The normalization of BMI in obese T2DM patients due to weight loss may also have a good impact on glycaemic management, which is among the goals that DM patients ought to strive towards (Harbuwono *et al.* 2021).

A noteworthy discovery was made by Firouzi *et al.* (2015) in their investigation: women had a smaller mean waist circumference than men. For men, a waist circumference of 90 cm was optimal, and for women, it was 80 cm (Sazlina *et al.* 2015), and studies have shown that Asian people, particularly South Asians, are more

Table 1. Nutritional assessment methods of T2DM patients in Southeast Asian countries

Author and year	Parameters and location	Study design and sample size	Population and age	Nutritional assessments			
				Anthropometry	Biochemical	Clinical	Dietary
Artha *et al.* 2019	Anthropometry, biochemical Indonesia	Retrospective study 140 patients	T2DM 30–65 years old	Weight, height, BMI	HBA1c, lipid profile; LDL-C, HDL-C, TG, and TC	-	-
Adrian & Maria 2022	Anthropometry, dietary Indonesia	Cross-sectional study 260 patients	T2DM ≥20 years old	Weight, height, BMI	-	-	1x24 hours dietary recall
Dalan *et al.* 2013	Anthropometry, biochemical Singapore	Cross sectional study 575 patients	T2DM 25–75 years old	Weight, height, BMI	FSG, HbA1c, serum creatinine levels	-	-
Fatmah 2020	Anthropometry, biochemical, clinical, dietary Indonesia	Quasi-experimental study 70 patients	T2DM 35–75 years old	Weight, height, waist and hip circumference, BFP	FBS, lipid profile; LDL, HDL, TG, and TC	Blood pressure	3-day dietary recall
Firouzi *et al.* 2015	Anthropometry, biochemical, clinical, dietary Malaysia	Clinical trial 104 patients	T2DM Mean age = 56.7±9.94 years old	Weight, height, BMI, waist circumference	FSG, HbA1c, plasma lipid components; TG, TC, and HDL	Blood pressure	3-day dietary recall
Harbuwono *et al.* 2021	Anthropometry, biochemical, clinical Indonesia	Prospective observational study 37 patients	T2DM 40–70 years old	Weight, height, BMI, waist circumference	FBG, HbA1c, lipid profiles; LDL, HDL, TG, and TC	Blood pressure	-
Hening *et al.* 2019	Biochemical, clinical Indonesia	Prospective quasi-experimental study 81 patients	T2DM ≥20 years old	-	HbA1c, FBG, PPBG, lipid profiles; LDL, HDL, TG, and TC	Blood pressure	-
Lam *et al.* 2015	Anthropometry, biochemical, clinical Singapore	Cross sectional study 1,891 patients	T2DM 21–74 years old	Weight, height, BMI, waist and hip circumferences, BAI, WHR, WHtR	FBG, lipid profiles; TC, HDL-C and TG	Blood pressure	-
Lim *et al.* 2015	Anthropometry Singapore	Cohort study 13,278 patients	T2DM	Weight, height, BMI, waist and hip circumference	-	-	-
Rusdiana *et al.* 2020	Anthropometry, biochemical, clinical Indonesia	Quasi-experimental study 40 patients	T2DM >40 years old	Weight, height, BMI, waist circumference	FBS and HbA1c	Blood pressure	-

Continue from Table 1

Author and year	Parameters and location	Study design and sample size	Population and age	Nutritional assessments			
				Anthropometry	Biochemical	Clinical	Dietary
Savira & Amelia 2018	Anthropometry, biochemical, clinical Indonesia	Quasi-experimental study 80 patients	T2DM >40 years old	Weight, height, BMI, waist circumference	FBS and HbA1c	Blood pressure	-
Sazlina *et al.* 2015	Anthropometry, biochemical, clinical Malaysia	Cross-sectional study 21,336 patients	T2DM ≥60 years old (range 60–104 years old)	Weight, height, BMI, waist circumference	HbA1c, fasting plasma lipids	Blood pressure	-
Thambiah *et al.* 2016	Biochemical Malaysia	Cross-sectional, retrospective study 214 patients	T2DM ≥20 years old	-	Lipid profiles; LDL, HDL, TG, and TC	-	-
Thewjitcharoen *et al.* 2018b	Anthropometry, biochemical, dietary Thailand	Cross sectional study 304 patients	T2DM 25–85 years old	Weight, height, and BMI	HbA1c, lipid profiles, and serum creatinine	-	3-day dietary recall

BAI: Body Adiposity Index; BFP: Body Fat Percentage; FBG: Fasting Blood Glucose; FBS: Fasting Blood Sugar; FSG: Fasting Serum Glucose; HbA1c: Glycated Hemoglobin; HDL-C: High Density Lipoprotein; LDL-C: Low Density Lipoprotein; WHtR: Waist-to-Height Ratio; PPBG: Postprandial Blood Glucose; T2DM: Type 2 Diabetes Mellitus; TC: Total Cholesterol; TG: Triglyceride Levels; WHR: Waist-to-Hip Ratio

susceptible to abdominal obesity because they are not regularly engaged in physical exercise (Firouzi *et al.* 2015). According to comparable data from diabetes patients in Kelantan, most diabetes patients do not engage in regular physical exercise (Firouzi *et al.* 2015). As the subjects of these studies were primarily overweight, obese, and had abdominal obesity, two studies from Indonesia (Fatmah 2020) and Singapore (Lam *et al.* 2015) reported that body fat percentage and Body Adiposity Index (BAI), respectively, were also included in the T2DM patients' assessments.

Monitoring metabolic indicators, such as body weight and body fat, is essential for the therapeutic care of patients with diabetes due to these patients frequently have hypertension, obesity, and dyslipidemia (Savira & Amelia 2018). BMI is the anthropometric indicator for which most research has been conducted. The conflicting results between obesity and overweight regarding T2DM-related mortality risk may be explained by the use of BMI alone, a broad indication of obesity that does not distinguish between fat and lean mass or depict body fat distribution (Lim *et al.* 2015). Lam *et al.* (2015) have come to similar findings and speculated that measures of central adiposity are good indicators of visceral adiposity and, as a result, are more closely associated with diabetes than BMI, which more accurately represents body volume and mass. In light of this research, it would be preferable to also use a mix of measurements, such as one that contains both a general and a central adiposity measure (Lam *et al.* 2015).

Furthermore, Lam *et al.* (2015) also stated that the BAI assessment may be useful in determining overall adiposity, although it is not expected to surpass BMI which demonstrates that the BAI operates similarly to BMI. As a result, the BAI has no added value once BMI has been taken into consideration (Lam *et al.* 2015). This is in line with the fact that while BAI makes an effort to estimate Body Fat Percentage (%BF), it does not account for how adiposity is distributed throughout the body. As a result, it would serve as an all-encompassing adiposity measure, similar to BMI. Therefore, Lam *et al.* (2015) asserted that there is no evidence to suggest that the BAI would be a better general measure of adiposity than the BMI; rather, it would serve the same purpose. Moreover, validation studies consistently show that the BAI tends to overestimate or underestimate adiposity at the extremes of BF (Lam *et al.* 2015).

However, according to a study from Singapore by Lam *et al.* (2015), BMI is unable to distinguish between lean mass and fat mass; as a result, changes in body adiposity with a given BMI across age, gender, and ethnicity confine it. Furthermore, some data suggests visceral adiposity, rather than total adiposity, is more directly associated with the metabolic side effects of obesity (Lam *et al.* 2015). This is a key fault in the BMI since it fails to account for body fat distribution. As a result, several adiposity metrics that take into consideration the distribution of body fat, such as Waist Circumference (WC), Waist-to-Hip Ratio (WHR), and Waist-to-Height Ratio (WHtR), have been devised and examined. In an adult population, a combination of BMI and WHtR may have the most therapeutic benefit, despite the apparent equality of the relationships between BMI, WC, WHtR, and diabetes (Lam *et al.* 2015).

Biochemical

The biochemical tests used were lipid profiles, Glycated Hemoglobin (HbA1c), and Fasting Blood Glucose (FBG), according to the articles we reviewed. Twelve out of the fourteen articles were evaluated, and six papers from Indonesia, three from Malaysia, two from Singapore, and one from Thailand were among them (Table 1).

The assessment of biochemical profiles was done, according to Savira and Amelia (2018), to ascertain the participants' level of diabetes management. A measure of blood glucose control during the previous three months was HbA1c, and at the time FBG demonstrated the significance of diabetes control. According to research by Firouzi *et al.* (2015), the individuals' average FBG and HbA1c were higher than what the treatment was supposed to achieve. HbA1c and fasting glycemia were only optimum in 28% and 20% of the individuals, respectively. Based on Malaysian diabetic patients' recommended levels, out of these subjects, only 20.2% and 27.9% met the recommended targets for FBG (FBG<7.0 mmol/L) and HbA1c (HbA1c<6.5%), respectively (Firouzi *et al.* 2015).

However, research from Thailand found that, based on having an HbA1C below 7.0%, more half of the individuals who were enrolled had adequate glucose control. This might be because the patients are already receiving a variety of teaching techniques, such as coaching, follow-ups, and motivational interviews, as needed to guarantee adherence and the intended results (Thewjitcharoen *et al.* 2018b). According to a study from Indonesia, obtaining effective glycemic control requires diabetes education, as HbA1c<6.5% was found to be an indicator for the control of T2DM in this study (Rusdiana *et al.* 2020). Since they only knew FBG as a control glycemic for DM, many people were unaware of the control glycemic of the HbA1c result.

The main objective of DM is blood glucose stabilization because DM has a significant negative impact on health due to its high morbidity and death rates. The American Diabetes Association (ADA) recommends glycemic control as one of the most important strategies for the management of type 2 diabetes (T2DM) since HbA1c is the best indication of glycemic level during the past three months (Rusdiana *et al.* 2020).

Nine out of the fourteen publications that were reviewed (four from Indonesia, three from Malaysia, one from Singapore and one from Thailand) also included results from lipid profile tests in their assessments. This is because obesity and dyslipidemia are prevalent in diabetes patients, thus monitoring metabolic indicators such as lipid profiles, FBG, and HbA1c is essential in the clinical treatment of diabetic patients (Savira & Amelia 2018). Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C), and Triglycerides (TG) were all included in the lipid profiles.

According to ADA guidelines, people with diabetes should have periodic serum lipid testing as a screening tool to identify the presence of dyslipidemia (Artha *et al.* 2019). The targets for lipid control were, according to Sazlina *et al.* (2015): (a) LDL-C: 2.6 mmol/L; (b) HDL-C:>1.0 mmol/L for males and >1.3 mmol/L for women; and (c) TG:<1.7 mmol/L. Thambiah et al. (2016) discovered that patients with HbA1c≥6.5% had significantly higher TC, TG, non-HDL, and TC/HDL ratios than patients with HbA1c<6.5%. The noteworthy correlation shown between dyslipidemia and glycemic state highlights the potential utility of HbA1c as a dyslipidemia biomarker (Thambiah *et al.* 2016). Thus, HbA1c can be employed as a biomarker in predicting dyslipidemia among T2DM patients in addition to glycemic management.

Clinical

Eight out of fourteen studies—five from Indonesia, two from Malaysia, and one from Singapore—include Blood Pressure (BP) in their analyses of T2DM (Table 1). Due to diabetes patients frequently have hypertension, monitoring metabolic indicators like BP is crucial for the clinical care of patients with diabetes (Savira & Amelia 2018).

Hypertension was defined as a systolic blood pressure measurement of more than 140 mmHg and a diastolic blood pressure measurement of more than 90 mmHg. Other studies have produced similar results, which are thought to be because BMI better reflects body volume and mass, which is associated with blood viscosity and blood volume and, therefore, more closely related to blood pressure, while measures of central adiposity are good indicators of visceral adiposity and, therefore, more closely associated with diabetes (Lam *et al.* 2015).

A Malaysian investigation discovered a link between high BP and inadequate glycemic management. This finding was consistent with that of another study, which found that those with T2DM who had uncontrolled hypertension had a higher likelihood of having poor glycemic control compared to those with normal blood pressure (Sazlina *et al.* 2015). Although it has been demonstrated that elderly individuals can benefit from lowering their BP, it should be emphasized that they have decreased tolerance; as a result, the treatment must be started gradually (Sazlina *et al.* 2015).

Hening *et al.* (2019) asserted that preventing the onset of diabetes complications like cardiovascular disease requires a thorough reduction in risk variables other than blood glucose, such as BP and lipid control. To sum, BP is a useful indicator for diabetes patients in preventing the onset of cardiovascular disease and other diabetes complications like hypertension. Additionally, because diabetes patients with uncontrolled hypertension typically have poor glycemic control, BP is also linked to poor glycemic control (Hening *et al.* 2019).

Dietary

Four out of the fourteen publications—two from Indonesia, one from Malaysia, and one from Thailand—incorporated dietary assessments in their research (Table 1). Based on the findings, the dietary assessment tools included were the 3-day diet recall, and 24-hour diet recall. According to Fatmah (2020), patients with DM are advised to limit their daily consumption of the seven nutrient groups—carbohydrates, protein, fat, vitamins, minerals, dietary fibre, and water. One of the various tools that may be used to assess eating patterns is the 24-hour dietary recall. Other tools include the Food Frequency Questionnaire (FFQ), diet histories, and dietary records.

The dietary record and 24-hour dietary recall are both entirely open-ended questionnaires that gather a wide range of specific information about the food consumed during a predetermined period. A Thai study (Thewjitcharoen *et al.* 2018b) found no association between total energy and macronutrient intake between patients who achieved appropriate glycaemic control (HbA1C<7.0%) and patients who did not (HbA1C>7.0%). In addition, a Malaysian study revealed that the majority of participants (62%) had abdominal obesity besides being overweight and obese overall. According to a 3-day dietary recall used in this study, the respondents had lower intakes of fibre (10.6±5.8 g), calcium (629±314 mg), and vitamin C (60.6±55.7 mg) which was recommended for people with T2DM (fibre: 20–30 g; calcium: 800–1,000 mg; vitamin C: 70 mg) (Firouzi *et al.* 2015).

A straightforward 24-hour dietary recall carried out by qualified dietitians could, in contrast to 3-day dietary data, provide accurate estimates of food groups such as energy, macronutrient intakes, and fibre, according to Thewjitcharoen (2018a). However, it proved inadequate for estimating data at the individual level. Large cross-sectional surveys usually utilize the 24-hour dietary recall, although prior research from Western populations consistently shows that food intake is underreported in dietary recall (Thewjitcharoen 2018a). Furthermore, concentrating on food-based healthy eating habits rather than employing a single nutrient-based assessment could help predict diabetic patient outcomes more accurately (Andriani & Maria 2022).

Strength and limitations

The comprehensive electronic search conducted for this study utilising Google Scholar, Science Direct, PubMed, and Scopus as four databases and a broad range of search terms to find as many linked articles as possible is one of this study's strongest points. We were also able to examine the nutritional assessment methods thanks to this scoping review that was already in use among T2DM patients, spot any gaps, and propose possible paths for method improvement

However, there are some limitations to this scoping review. This scoping review, in contrast to a systematic review, does not assess the calibre of the selected papers. Furthermore, unpublished research that has not been made available online may indicate a lack of nutritional assessment data. Due to the small number of studies that satisfied the inclusion and exclusion criteria, variations in sample sizes, participant restrictions, reference standards, and geographic locations, it was challenging to generalise the findings. Another issue with the review is that it only discovered relevant research in four of the eleven Southeast Asian countries which are Indonesia, Malaysia, Singapore, and Thailand.

CONCLUSION

This review examined the available nutritional assessment technique that is used for nutritional assessments of people with T2DM in Southeast Asian countries. According to the research, nutritional assessment techniques were most commonly applied in these areas: anthropometry, biochemical, clinical, and dietary. The parameters that were frequently measured in anthropometric assessments were weight, height, BMI, waist and hip circumference, and body fat percentage. Biochemical assessments mostly utilized were FBG, HbA1c, and lipid profiles to assess the glycaemic value and cholesterol level of the patients. Some studies also took Blood Pressure (BP) for clinical assessments, while the tools usually utilised for dietary assessments are 3-day and 24-hour dietary recalls.

Hence, it is crucial to ensure that the nutritional assessment procedures in those countries are frequently revised to efficiently handle patients with diabetes. Even though the majority of the results used comparable parameters, only one study included BAI in their research. Although it was claimed that the BAI would be equally useful as the BMI as a general predictor of adiposity, however there was no evidence to support its superiority. Therefore, more research is required to confirm the parameter's suitability for T2DM patients.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Universiti Sains Malaysia Short-Term Grant 304/PPSK/6315577 for providing support for this study.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare that no conflicts of interest arose during any part of the research process.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan **J. Gizi Pangan, March 2024, 19(1):21-30**
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2024.19.1.21-30*
ISSN 1978-1059 EISSN 2407-0920

Physicochemical Properties, Glycaemic Index and Glycaemic Load of Chocolate Energy Bars Prepared with High Polyphenols Cocoa Powder and Guar Gum

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ABSTRACT

This study aimed to determine the physicochemical properties, Glycaemic Index (GI) and Glycaemic Load (GL) of chocolate energy bars prepared with different percentages of guar gum (0.5%, 1.0%, 1.5%) and high polyphenols cocoa powder. Proximate analysis was determined following the official methods Association of Official Analytical (AOAC). Ten eligible respondents who met the inclusion and exclusion criteria participated in the GI and GL determination. Test and reference food were given to the participants after overnight fasting. Blood capillary finger pricks were drawn for 7 interval time at 0 until 120 min. The blood glucose responses were calculated based on Incremental Area Under Curve (IAUC). The result revealed that the fat and calorie content were significantly lower in formulations with highest guar gum percentage ($p<0.05$). On the other hands the moisture, ash, carbohydrate contents, crude fibre, energy value, hardness, fracturability, pH value, and colour were all comparable to the control sample. The control and chocolate energy bars with 0.5% guar gum were in the category of high GI and GL. Whereas, the chocolate energy bars with 1.0% and 1.5% guar gum were in the medium category for both GI and GL. This study demonstrated that incorporating different percentages of guar gum in the high polyphenols chocolate energy bar changed some of the proximate compositions but not the physical properties. Furthermore, addition of guar gum affected the GI and GL as the values were reduced with the increased amount of guar gum.

Keywords: dark chocolate, guar gum, physicochemical properties, glycaemic index, glycaemic load

INTRODUCTION

Chocolate is a popular and enjoyable food item consumed by millions of people due to its distinct, rich, and sweet flavour. Malaysians consumed more chocolate than other region residents. The report stated that the average Malaysian consumed 0.5 kg of chocolate annually, whereas South-East Asian neighbours, such as the Philippines and Indonesia, consumed lesser with 0.3 kg, respectively (Durai 2022). The three most common commercial chocolate frequently seen in grocery shops are white, dark and milk chocolate. In comparison to milk and white chocolate, dark chocolate has the most considerable cocoa content. Most dark chocolate contains more cocoa and less sugar. Nutritionally, dark chocolate products have gained a positive reputation for their health benefits. Dark chocolate contains several health promoting elements such as bioactive components like polyphenols, flavonoids, procyanidins, theobromines, as well as vitamins and minerals that positively modulate the human immune system (Samanta *et al.* 2022). The high antioxidant contents in dark chocolate contributes to a prominent regulatory role such as lowering blood pressure, bolstering the blood flow and maintaining the immune system (Latif 2013).

High cocoa content in chocolate showed a strong correlation with content of dietary fibre and iron (Chen 2018). Hence, in addition to antioxidant, chocolate is rich in iron and dietary fibre. Iron is a vital nutrient that ensures proper growth development and functioning of the body and promotes the production of proteins that transport oxygen and regulate cell development. While, dietary fibre is known to have variety of health benefits, including treating colon disease, reducing the risk of heart disease, diabetes mellitus and colon cancer, improving blood sugar balance and digestive function.

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(Received 06-11-2023; Revised 12-01-2024; Accepted 01-02-2024; Published 30-03-2024)

(Barber *et al.* 2020). Dietary fibre from cocoa is derived from the cocoa bean seed coat or bran. (Sarriá *et al.* 2012). Soluble fibre also may help decrease cholesterol and assist control of blood sugar levels and the insoluble fibre is non-water soluble and bulks up the stool, hence reducing constipation (Newman 2020). In Europe, there is a special trend associated with consuming dark chocolate products with a high cocoa content, typically without the addition of milk (Alberts & Cidell 2006).

Food energy bars comprised mainly from cereals and other high-energy ingredients which are known as convenient snacks. The variable component in food energy bars are commonly carbohydrate, followed by protein, fat, and fibre (Gill & Singh 2020). Energy bars are now an acceptable option as a high-quality source of energy due to changes in dietary and lifestyle choices, increasing knowledge of nutrient-dense, healthy eating practices, and physical activity levels (Yadav & Bhatnagar 2015). Energy bars were initially advertised to athletes as a source of energy, but as the demand from luxury consumers increased and the number of customers who care about their health also increased, snack bar sales soared (Ho *et al.* 2016).

The energy bars may be a viable substitute for dietary supplements as it is further enriched with minerals, vitamins, phytochemicals, and other bioactive compounds. A higher Dietary Phytochemicals Index (DPI) is associated with decreased pre-diabetes (Davoudi-Kiakalayeh *et al.* 2017). Diets with a low Glycaemic Index (GI) are hypothesised to result in more stable blood sugar levels and weight loss by lowering postprandial glycemia (Ni *et al.* 2022). Low (GI) diets may also enhance endurance performance (Kaviani *et al.* 2019).

Polysaccharides could be used in place of cocoa butter to create low-fat chocolates (Amir *et al.* 2013). Pectin, xanthan gum, and guar gum were considered helpful in achieving the desired textural and organoleptic properties (Barman *et al.* 2021). Guar Gum contain high nutritious fiber content and excellent viscosity, that often used as an additive in food emulsifiers, as a thickener, and to solidify processed foods including breakfast cereal, dairy products, and baked goods (Aguilar *et al.* 2017). While it does not affect high-density lipoprotein or triglyceride levels, guar gum may lower total cholesterol and low-density lipoprotein levels. Therefore it may aid in maintaining healthy lipids and preventing Cardiovascular Disease (CVD) (Wang *et al.* 2021).

Hence, in this study aimed to develop chocolate bars prepared with different percentages of guar gum and high polyphenols cocoa powder to achieve better GI and GL index. In addition, the study also determined the proximate content, physical properties of chocolate energy bars prepared with different percentages of guar gum and high polyphenols cocoa powder to discover its potential as a functional food product.

METHODS

Design, location, and time

This descriptive study was conducted from 16th September 2022 until 10th February 2023. The chocolate energy bars were developed in the Food Preparation and Therapeutic Diet Lab and other analysis were carried in the respective lab of Universiti Sultan Zainal Abidin (UniSZA) campuses. This study was approved by the Universiti Sultan Zainal Abidin Human Research Ethics Committee (UniSZA/UHREC/2022/408).

Materials and tools

All raw materials and ingredients were purchased from the supplier (Callebaut Chocolate Finest Belgian Dark Chocolate) and other ingredients were purchased from the grocery market in Kuala Nerus, Terengganu, Malaysia. Glucometer Accu-Chek® Safe-T-Pro Uno (New South Wales, Australia) and glucose strips were used in collecting data for GI.

The chocolate energy bars were developed based on the formulation by Chandegara *et al.* (2018) with slight modification. It contains raw materials such nuts (almonds, cashew nut), dates (Phoenix dactylifera), raisin, rice crispies, oats (Avena sativa), honey (multiflora), seeds (sunflower and watermelon), cocoa powder. Three different percentages of guar gum at 0.5%, 1.0% and 1.5% were used to formulate the chocolate energy bars. All the ingredients were weighed according to the formulations (control) and were interchanged for other formulations of 0.5%, 1.0%, and 1.5% of guar gum respectively. Prior to the analysis, the energy bar was stored in the chiller with temperature of 4°C.

Procedures

Proximate analyses. The moisture, ash, crude protein, fat, and fibre content of the chocolate energy bars were determined based on standard procedures of AOAC International method. All analyses were conducted in triplicates.

Total carbohydrate and energy estimation. The total carbohydrate content was calculated by difference [Total Carbohydrate (% wet basis)=100% - % (moisture content + ash + crude protein + crude fat + crude fibre]. The energy content of the sample was estimated by multiplying by the factor values; [1 g of crude protein or carbohydrates provides 4 kcal and 1 g of crude fat provides 9 kcal of energy] (Said *et al.* 2019).

pH value measurement. The acidity and alkalinity were assessed using a pH meter (Mettler Toledo, Ohio, USA) from 5 g samples finely mixed and homogenized with 20 mL of distilled water.

Texture profile analysis (Hardness and fracturability). The hardness and fracturability of the energy bar according to mechanical characteristics was analyzed using a TA.XT.Plus texture analyzer (Stable Microsystems, UK). The 3-point bend rig probe was positioned centrally beneath the sample, and it was moved until the probe made contact with the sample before the deformation curves were captured (Mamat *et al.* 2018).

Colour profile analysis. The colour profile analysis was conducted using the Konica Minolta colorimeter (Konica Minolta, Tokyo, Japan) based on the L^* (lightness), a^* (redness), and b^* (yellowness) colour system. Colorimeter was calibrated prior to use with a white calibration plate. The instrument was positioned on top of the energy bar formulation, and each energy bar's L^* , a^*, and b^* values were recorded and analysed (Zainol *et al.* 2020).

Glycaemic index and glycaemic load determination. The value of GI for each sample was determined by the following protocol from the International Organisation of Standardisation ISO 26642:2010 (International Organisation for Standardisation 2010 Food products: Determination of the Glycaemic Index (GI) and recommendation for food classification). Ten eligible subjects with consent form that fulfilled the inclusion criteria, were selected to participate in this study. The screening procedures were followed to the standard guidelines for GI testing. All subjects underwent an Oral Glucose Tolerance Test (OGTT) before the GI test to certify their both inclusion and exclusion criteria.

The age of subjects ranged from 19–45 years old with normal Body Mass Index (BMI) and normal glucose tolerance. The inclusion criteria include healthy and not under any treatment or medications. Additionally, participants must be three months free from any clinical trial and were not allergic to the ingredients such as nuts, cereals, chocolates, and guar gum. The subjects were given 50 g of reference food and all three chocolate samples (0.5%, 1.0% and 1.5% of guar gum) within 3-days washing period for each food/bar. The blood glucose were taken for 2 h (0 min, 15 min, 30 min, 45 min, 60 min, 90 min and 120 min). The GI of chocolate energy bars was calculated by following the World Health Organization/ Food and Agriculture Organization (WHO/FAO1998) recommendation. GI and GL values were calculated as the following:

$$\text{GI} = \frac{\textit{Incremental Area Under Curve (IAUC)of each chocolate energy bar}}{\textit{Average IAUC of the reference food (white bread or glucose)}} \text{ x } 100$$

$$\text{GL} = \frac{\textit{GI of carbohydrate x grams of carbohydate per serving food}}{100}$$

Data analysis

All data were analyses using the SPSS software, version 22 (IBM, Corp, Chicago, USA). The results of proximate analysis from the triplicate samples of each chocolate energy bar type were compared using the one-way of variance (ANOVA). The Tukey post hoc test was performed to determine the significance difference at $p<0.05$. Data regarding GI and GL were presented in descriptive statistics.

RESULTS AND DISCUSSION

Proximate composition

As shown in Table 1, the moisture content of chocolate energy bar prepared with different percentages of guar gum varying from 9.43% to 10.53%. Albeit, the highest moisture content was found in the 1.5% guar gum formulation, it was comparable to the control ($p>0.05$). The lowest moisture content was indicated at 9.43% for the 0.5% gur gum formulation. This was likely due to the hydrophilic polymers in guar gum that prevent moisture loss in the food products (Nehra

Table 1. Proximate composition of chocolate energy bars prepared with different % of guar gum

Components (%)	Chocolate energy bars with different % of guar gum			
	0	0.5	1.0	1.5
Ash	2.33±0.03[a]	2.38±0.01[b]	2.33±0.01[a]	2.34±0.01[a]
Moisture	10.12±0.13[b]	9.43±0.09[a]	9.92±0.14[a]	10.53±0.11[b]
Fat	15.05±0.20[b]	14.72±0.38[ab]	14.20±0.34[ab]	13.91±0.67[a]
Fibre	3.50±0.04[a]	3.76±0.28[a]	3.81±0.25[a]	3.98±0.21[a]
Protein	11.04±0.36[a]	11.88±0.00[a]	10.83±0.36[a]	10.42±0.36[a]
Carbohydrate	61.86±0.54[a]	62.31±0.56[a]	62.40±0.74[a]	62.77±1.19[a]
Energy (kcal)	427.05±1.41[c]	429.24±3.11[c]	420.72±0.51[b]	417.92±5.72[a]

[abc] means in the same row with different lowercase letters are significantly different ($p<0.05$)

et al. 2022). Goswami *et al.* (2019) reported that moisture content had increased as the guar gum percentage in the product increased. This might happen due to the carbohydrate-based hydrocolloids properties, including guar gum possess higher water-holding capacity to retain the moisture in the food matrix (Colla *et al.* 2018).

The lowest fat content was found in chocolate energy bars with 1.5% guar gum (13.91 g/100 g) while the highest fat content was in the control (15.68 g/100 g). This because guar gum substitutes the fat content (Bavaro *et al.* 2021). Samakradhamrongthai *et al.* (2022) stated that substitution of guar gum and polydextrose, decreased fat content by 27.86% in comparison to the control formulation. Carbohydrate-based hydrocolloids is a potential fat replacements since they are water-soluble and hydrophilic by nature (Colla *et al.* 2018). The structure of Carbohydrate-based hydrocolloid can provide both full and partial functions of fat while offering lower fat calories (Kumar 2021).

In comparison, there was a decrease in protein content for control chocolate energy bars with 1.0 % and 1.5% guar gum formulation with no significant differences ($p>0.05$). The 0.5% guar gum formulation had the highest protein (1.88%) while 1.5% guar gum ranked the lowest (10.42%). Contradict to a study conducted by Goswami *et al.* (2019), the protein content increased when the guar gum percentage in the product increased. This can be due to the control chocolate and 0.5% guar gum had the highest amount of oats compared to other samples.

The highest fibre content was found in the 1.5% guar gum formulation (3.98%) and the lowest was found for the control (3.5%), but the difference was not statistically significant. On average, processed guar gum products contain a crude fibre content ranging from 1.61%–2.5% (Farah *et al.* 2016). The study was supported by Goswami *et al.* (2019) indicating that an increment in fibre content was related to an increased percentages of guar gum in the product. However, in our study the control chocolate energy bars had the highest amount of oats as compared to other samples. As reported by Munir *et al.* (2018), the addition of oat also increased the fibre constituents in snack bars. Hence, there result was not statistically significant.

The ash content of the chocolate energy bar was comparable in all formulations (ranging from 2.33%–2.48%), respectively. This is supported by a study by Farah *et al.* (2016) that reported the processed product of guar gum contains small amount of ash. Furthermore, several studies also reported that the ash content increased with the higher added guar gum incorporated in the composite flour and carabeef cookies (Immanuel & Singh 2022). However, according to the National Germplasm Resources Laboratory (USDA 2009), the other possible explanation for the comparable ash content in this study was likely due to the nuts that were used, as almonds have higher ash content (3.13%) and they were considered as the source of minerals including magnesium, calcium, and iron (Fernandes *et al.* 2013).

Carbohydrate content was also comparable with no significant difference (p>0.05) between all dark chocolate formulations. The range of carbohydrate content was from 61.86% to 62.77%, while in the control energy bar was 62.31%. The study conducted by Nazira and Azada (2016) reported that the carbohydrate content was increased when the guar gum percentage in the product increased. However, in this study, the carbohydrate content was not significantly varied because the major carbohydrate sources in this formulation are mainly nuts and honey.

Table 1 indicates that there was a significant difference in the amount of calories between the formulations. The energy value per 100 g ranged from 417.92 kcal to 429.24 kcal. The 0.5% formulation sample contained the highest calorie content (429.24 kcal) and the second was the control (427.05 kcal) with no significant difference. While the formulation of 1.5% guar gum exhibited the lowest calorie value (417.92 kcal). On average, these results were lower than the study by Tiwari *et al.* (2017), as a 100 g energy bar contained 440 kcal–500 kcal. The study by Sharma *et al.* (2021) produced multi-seed energy bars for sports persons with an energy value of 444.66 kcal per 100 g. In this study, the decrease in energy bar caloric value could be due to the lower amounts of cereals, nuts and fat used in the bars (Eke-Ejiofor & Okoye 2018) which were substituted with the guar gum.

The texture of chocolate energy bar (hardness and fracturability)

Table 2 presents the texture of chocolate energy bars incorporated with different percentages of guar gum. The addition of guar gum in a formulation is associated with lower hardness score. As shown in research by Amir *et al.* 2013, who reported that hardness decreased with the increasing level of guar gum. However, the result in this study showed inconsistencies in findings. The addition of 0.5% and 1.0% of guar gum resulted in lower hardness score. But there is an increase in hardness in the 1.5% guar gum formulation, which was higher than the control.

This was likely caused by other unbridled factors, such as the irregular arrangement of ingredients of energy bars, such as cashew nuts, almonds, pumpkin seeds, and sunflower seeds. There were sections of the chocolate energy bar that contained those hard ingredients and vice versa, which caused the cutting blade to cut irregularly. However, an alternative explanation can be associated with the characteristic of guar gum which absorb more water resulted in a harder gluten network and increase the hardness value (Goswami *et al.* 2019). This was consistent with the study of fat substitution (xanthan and guar gum) that increased the hardness of baked and pastry products (Aggarwal *et al.* 2018).

The fracturability of chocolate energy bars with various amounts of guar gum showed variations in fracturability. However, the difference between treatments (control, 0.5%, 1.0%, and 1.5%, respectively) was not statistically significant. The addition of more gums and carbohydrate-hydrocolloid-based ingredients increased the hardness of the cookies while decreasing their fracturability (Hussain *et al.* 2022). The study conducted by Singh *et al.* (2015) was also in accordance with this study, whereby the fracturability was decreased when the guar gum percentage in the product increased. However, the fact that there were sections of the chocolate energy bar that contained inconsistent ingredients might contribute to this variations in results.

Table 2. Physical properties of chocolate energy bars prepared with different % of guar gum

Formulation (%)	Texture value		pH	Colour value		
	Hardness	Fracturability		L^*	a^*	b^*
0	1.17±0.67[a]	1.19±0.31[a]	6.46±0.11[a]	36.25±1.75[a]	6.23±1.02[a]	10.38±1.56[a]
0.5	0.74±0.83[a]	1.00±0.3[a]	6.61±0.02[a]	31.06±5.73[a]	7.55±1.81[a]	9.93±1.38[a]
1.0	0.50±0.63[a]	0.84±0.31[a]	6.64±0.09[a]	33.16±2.70[a]	7.77±0.54[a]	10.89±0.87[a]
1.5	1.60±1.09[a]	1.19±0.62[a]	6.68±0.14[a]	30.74±2.18[a]	8.88±1.53[a]	11.41±1.52[a]

[abc] means in the same column with different lowercase letters are significant different (p<0.05)

pH value

The pH value of chocolate energy bars incorporated with different percentages of guar gum showed an ascending scale ranging from pH 6.46 (control), pH 6.61 (1.0% guar gum), pH 6.64 (1.5% guar gum) and pH 6.68 (2.0% guar gum), respectively. But the difference was not statistically significant. The pH value for all samples were found to near neutral with slight variation in the reading. The study by Nazira and Azada (2016) also showed similar outcomes, whereby the pH value increased when the guar gum percentage in the food products increased.

Colour profile

Colour attributes are one of the main factors that are perceived and impacted people's attitudes when choosing the food products. Chocolate composition, chocolate processing methods and roughness can affect the chocolate colour as the appearance of dark chocolate is regulated by its shape, shininess, surface appearance and smoothness or roughness (Saputro *et al.* 2017). In this study, all the colour values were observed and showed no significant differences amongst the treatments. The lightness (L^* value) for the chocolate energy bar (1.0%) was higher than the others (0.5% and 1.5% guar gum). Besides, there was an increment of the redness (a^* value) and yellowness (b^* value) from the control to the 1.5% guar gum chocolate energy bars. The lightness and yellowness results were in line with a study by Barman *et al.* (2021), whereas the redness was dissimilar. The addition of guar gum in the food products, including pastries affect the final colour because guar gum can react in partial hydrolysis of the mono- and disaccharides (Culetu *et al.* 2021). However, no significant differences were recorded between treatments for all the values (lightness, redness, and yellowness). Similar findings was reported by Goswami *et al.* (2019) that developed carabeef cookies with different percentages of guar gum, and it showed no significant differences in the values of redness, yellowness, chroma, and hue angle values between control and the formulated carabeef cookies. This condition might be caused by adding the guar gum in a very small amount and water is used to maintain the consistency of the formulation. Kamal (2015) developed low-calorie fibre-enriched biscuits supplemented with rice bran and guar gum and observed no significant colour differences.

Glycaemic index and glycaemic load evaluation

The meta-analysis study by Greenwood *et al.* (2013) reported that for every 50 g of carbohydrates consumed, the relative risk of Type 2 diabetes is 0.97. In this study, 50 g of carbohydrates was used as the dose-response curve of the IAUC glucose response has been shown to be in linear within the range of 25 g to 50 g of carbohydrates samples. Figure 1 illustrates the results of GI and GL of different percentages of guar gum incorporated in the chocolate energy bar. The chocolate energy bars with 0.5% guar

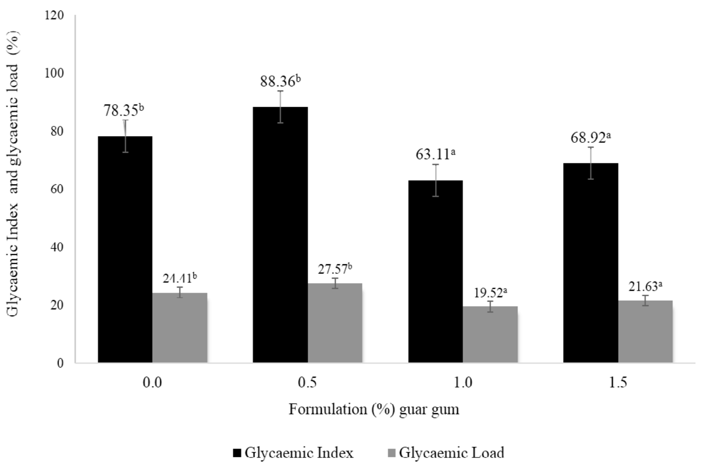

Figure 1. Glycaemic index and glycaemic load of chocolate energy bars prepared with different % of guar gum

gum had the highest GI value (88.35), value as compared to the control (78.35), 1.5% guar gum (68.92) and the lowest GI value was 1.0% guar gum (63.11) ($p<0.05$). Both control and 0.5% were in the high GI category, meanwhile, 1.0% and 1.5% guar gum formulations were in the medium. Theoretically, the GI value is decreased in parallel with the higher addition of guar gum as guar gum can reduce the absorption of sugar and lead to a decreased in blood sugar levels due to its characteristic as soluble fibre.

Functional components with a GI-lowering impact, including soluble dietary fibres and hydrocolloids like guar gum, aids in lowering the rate of carbohydrate hydrolysis and reduce the GI of diets (Santamaria *et al.* 2023). A study with 10% of guar gum in noodles demonstrated a low GI value (46.2) and possessed a high GL (Kumar & Prabhasankar 2016). Previous study on guar gum in the bread formulation has observed hypoglycaemic effect, effectively able to decrease in starch digestibility and reduce post-prandial glycaemic responses (Maehre *et al.* 2021).

Honey, such as added to this energy bar formulation, potentially containing additional sugar ingredients, that results in a higher GI value. Some honey in the market was incorporated with sugar as common ingredients including the sugar solutions from corn syrup and cane sugar syrup (Nik Husain & Ghazali 2021). Despite honey is mostly made up of different simple sugars, it has similar glycaemic response as glucose (Rajab *et al.* 2017).

The GL of food is an estimation of how much food will elevate a person's blood glucose level after consuming it. One gram of glucose consumption will increase the blood glucose to approximately one unit of GL (Kumar & Prabhasankar 2016). This decrease in GL associated with increasing guar gum percentage can be attributed to the reduction in the GI, as it follow a similar pattern. It was postulated that the reason of differences in GI for the formulations is the cocoa biochemical contents, which are a rich source of high-quality antioxidant polyphenols. They mainly comprise anthocyanins (4 % of total polyphenols), catechins (29%–38% of total polyphenols), and proanthocyanidins (58%–65% of total polyphenols) (Aprotosoaie *et al.* 2016). The dietary polyphenols including anthocyanins and catechins found could reduce starch digestion by the inhibition of α-amylase and α- glucosidase resulting in a lower GI of foodstuffs (Uğur *et al.* 2022). Another study by Kawakami *et al.* (2021) showed that consumption of chocolate containing cocoa polyphenols before a 50 g OGTT could improve early insulin and Glucagon-Like Peptide 1 (GLP-1) secretion in healthy participants, hence illustrating the potential of chocolate containing cocoa polyphenols to control postprandial glucose excursions.

CONCLUSION

The result revealed that the fat and calorie content were significantly lower in formulations with highest guar gum percentage ($p<0.05$). On the other hands the moisture, ash, carbohydrate contents, crude fibre, energy value, hardness, fracturability, pH value, and colour were all comparable to the control sample. The control and chocolate energy bars with 0.5% guar gum were in the category of high GI and GL. Whereas, the chocolate energy bars with 1.0% and 1.5% guar gum were in the medium category for both GI and GL. The values were lower than the control and 0.5% guar gum formulations, which had higher GI and GL.

ACKNOWLEDGEMENT

This research was supported by Ministry of Higher Education (MOHE), Malaysia through Fundamental Research Grant Scheme (FRGS/1/2021/SKK05/UNISZA/02/1). The authors also would like to thank the Faculty of Health Sciences and the Center for Research Excellence and Incubation Management (CREIM), UniSZA.

DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 DOI: https://doi.org/10.25182/jgp.2024.19.1.31-40
ISSN 1978-1059 EISSN 2407-0920

Intervention with Purple Okra Pudding and Supplement to Improve Antioxidant Status in Healthy Adults

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ABSTRACT

This research aimed to analyze the potency of purple okra-based products in improving the antioxidant status of healthy adults. Thirty adults with high body fat percentages were allocated into three groups: the first group was treated with 100 g of purple okra pudding/day, the second group was provided with a purple okra extract supplement that contained 3.80 g of extract/day, and the third group was a control group. The intervention was carried out for 28 days. All subjects were exposed to nutrition education, and data on their characteristics, food intake, and physical activity level were collected. The results showed that purple okra pudding and supplement had antioxidant activity of 0.39 and 455.39 mg AEAC/g extract, IC_{50} of 543.79 and 71.78 ppm, and total phenol of 6.21 and 24.49 mg GAE/g extract, respectively. There were significant differences among subject groups in energy, protein, and fat intake as well as physical activity level. The group treated with purple okra pudding showed a significantly higher ΔSOD after the intervention, most probably due to the role of antioxidants contained in purple okra in upregulating antioxidant defense. In contrary, there was a declining trend of ΔSOD in the group treated with purple okra extract supplement. The different effects observed between the two groups might be due to the different phenol contents between the two intervention products. This study showed that purple okra has the potential as a functional food and health supplement in improving the antioxidant status of healthy adults with high body fat percentages as indicated by a higher change of SOD level (0.08 u/mL) in comparison to the control (-0.07 u/mL).

Keywords: antioxidant, pudding, purple okra, superoxide dismutase, supplement

INTRODUCTION

Oxidative stress is a cellular condition that occurs as a result of a physiological imbalance between Reactive Oxygen Species (ROS) production and endogenous antioxidant defenses (Ighodaro & Akinloye 2018). Free radical molecules, particularly ROS, are derived from biotransformation of molecular oxygen and external factors, including unhealthy eating patterns, lack of exercise, and exposure to chemicals from various sources (Sharifi-rad *et al.* 2020). Increased oxidative stress is associated with high body fat percentage and several degenerative diseases that have become one of the highest causes of death (Fernández Sánchez *et al.* 2011; Ighodaro & Akinloye 2018; WHO 2018).

The interplay between the body's natural antioxidant defenses and free radicals is important in maintaining health, prolonging aging, and preventing age-related diseases. Superoxide Dismutase (SOD) is one of the three key enzymes and first-line defense antioxidants that acts to suppress or prevent the formation of free radicals in cells. SOD works by breaking down hydrogen peroxides and hydroperoxides into harmless molecules and neutralizing molecules that have the potential of developing into free radicals with the possibility to induce other radical production (Ighodaro & Akinloye 2018; Liu *et al.* 2023).

Efforts to maintain and increase the activity of endogenous antioxidants such as SOD are expected to reduce oxidative stress. Apart from that, consumption of exogenous antioxidants, such as those contained in antioxidant-rich foods or supplements, will increase the total antioxidants that work to combat oxidative stress in the body so that optimal oxidative status can be obtained (Ighodaro & Akinloye 2018). Among the antioxidant-rich foods, okra (*Abelmoschus esculentus* L. Moench) is one of the vegetables widely studied for its bioactive components (Tyagita *et al.* 2019).

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(Received 19-10-2023; Revised 29-01-2024; Accepted 12-02-2024; Published 30-03-2024)

Okra, also known as lady's finger, is a type of vegetable that is popular in many parts of the world. It is usually green in color; however, there is also a purple variety known as purple okra (Manickavasagam *et al.* 2015). This vegetable contains bioactive compounds such as flavonoids and polyphenols, which have the potential as antioxidant and anti-inflammation (Majd *et al.* 2019). Biofortified purple okra is a superior variety identified as having higher amount of anti-diabetic bioactive components as compared to green okra (Anjani *et al.* 2018). Seventy percent of the antioxidant activity of purple okra comes from quercetin, which is the main flavonoid in purple okra, with a content of 0.45 mg g^{-1} (Roy *et al.* 2014; Anjani *et al.* 2018).

The extract of okra can be administered as an encapsulated supplement. The capsule protects the active substances contained from external factors and controls their release to the digestive system, thereby maintaining their functional stability. Purple okra extract has an antioxidant capacity of 4.17 mg AEAC g^{-1}, antioxidant activity (IC_{50}) of 316.86 ppm, total phenol content of 3.60%, and quercetin of 0.45 mg g^{-1} (Anjani *et al.* 2018). In extract form, it has more bioactive components, thereby allowing for a higher antioxidant content (Susanty & Bachmid 2016).

Bioactive components in purple okra can also be delivered in the form of pudding, which is used as a functional food. Pudding is usually consumed as a dessert and is well accepted because of its sweet taste and soft texture (Darmawan *et al.* 2014). Okra produces mucilage that also has an antioxidant activity and hydrocolloid properties that can act as a gelling agent in improving food texture (Cahyana *et al.* 2017). Measured by the DPPH method, a pudding with popping boba made from purple okra has an antioxidant capacity of 3.54 mg AEAC 100 g^{-1} (Dyastasari 2019). This research aimed to analyze the potential of purple-okra-based products in improving the antioxidant status of healthy adults.

METHODS

Design, location, and time

This study encompassed a laboratory phase and a quasi-experimental research segment employing a pre-posttest control parallel group design. Ethical clearance was obtained from the Commission on Ethics for Research Involving Human Subjects, IPB University, as per letter number 762/IT3.KEPMSM-IPB/SK/2022. The research was carried out of the IPB University, Darmaga campus, from November 2022 to April 2023.

Sampling

Subjects were students and staff of IPB University at Darmaga campus. The inclusion criteria were being in the adult age group (19–44 years), having a high Body Fat Percentage (BFP) of >28% for women and >20% for men, as measured with InBody 270 (Lee & Nieman 2007)), having a normal nutritional status (BMI 18.50–25.00 kg/m^2), not suffering from degenerative diseases as diagnosed by medical doctor, not smoking and consuming alcohol, not being pregnant or breastfeeding, having normal Fasting Blood Glucose (FBG) levels (70–100 mg dL^{-1}), having no milk allergy or intolerance, not currently taking antioxidant supplements and drugs, and willing to participate in the study by filling out the informed consent form.

Body fat percentage is directly related to higher markers of oxidative stress and lower antioxidant defenses (Fernández-Sánchez *et al.* 2011). We expected that those with high body fat percentages would be more responsive to antioxidants as given in the intervention.

The minimum number of subjects required was calculated using a sample size formula by Notoatmodjo (2012) as follows:

$$n = \frac{2\sigma^2 \times [Z_{1-\alpha/2} + Z_{1-\beta}]^2}{(\mu_1 - \mu_2)^2}$$

$$n = \frac{2(5.95)^2 \times [1.96 + 1.28]^2}{(85.38 - 76.19)^2}$$

$$n = \frac{2\,x\,35.4025 \times 10.496}{84.4561}$$

$$n = 8.79 \approx 9$$

Where n is the minimum sample size required, $Z_{1-\alpha/2}$ is the level of significance (Z=1.96, with a confidence interval of 95%), $Z_{1-\beta}$ is the test power (Z=1.28 with test power of 90%), σ is the standard deviation of the difference in mean SOD levels (Rosidi 2014), and μ1–μ2 is the significant difference in mean SOD levels between two groups (Rosidi 2014). With an additional 10% of dropout possibility, the minimum number of subjects required was 10 per group. Subjects were divided into three groups, namely: 1) group that received a purple okra supplement that contained purple okra extract at 3.80 g/day; 2) group that was given 1 cup (100 g) of purple okra pudding per day, and; 3) control group.

Research stages. This research consisted of two stages, namely preliminary research and main research. The preliminary research began with the production of supplement and pudding from biofortified purple okra, which was grown at the Leuwikopo Experimental Farm of IPB University.

The purple okra supplement was prepared by extraction using 96% food-grade ethanol (3:1) for 3×24 hours and drying using a vacuum pan evaporator (60°C, 30 minutes). The dried okra extract was then powdered and packaged into capsules.

The preparation of the purple okra pudding used a boiling method, which began with blanching (at 97°C for 30 seconds) of freshly-washed purple okra. The blanched okra was then extracted using water as the solvent (ratio 1:3) at room temperature for 12 hours in order to get its mucilage (Cahyana *et al.* 2017). The other parts of the blanched okra was then pureed using a blender until it was smooth. The okra mucilage and pureed okra were next mixed with the other ingredients including water, skimmed milk, agar powder, lemon and dragon fruit extracts, sorbitol, and vanilla essence. The products were analyzed for total phenolic content, antioxidant capacity, and antioxidant activity.

The main research was a clinical trial. Subjects were determined by screening according to the inclusion criteria and divided into three groups purposively by considering the subject's ownership of a refrigerator for pudding storage and residence. The next step was blood collection and measurements of SOD and FBG before the intervention, followed by an intervention period of 28 days according to Basu *et al.* (2021) and Subawa *et al.* (2022) on human subjects that showed improvement in oxidative markers after 28 days of intervention. During the intervention, nutrition education was provided in week 1, while 2×24 hour food recall and physical activity data collection was performed in weeks 1–4. Blood collection and post-intervention SOD level measurement were conducted on the 29th day.

Data collection

Data was collected by laboratory analyses of total phenolic content using the Folin-Ciocalteu method, antioxidant capacity using the DPPH method with ascorbic acid as the standard, and antioxidant activity using the DPPH method. Meanwhile, data collected from the subjects includes subjects' characteristics (Body Mass Index and BFP) that was measured using a digital body scale, a stadiometer, and a Bioelectrical Impedance Analysis (BIA), as well as their dietary intake and physical activity, obtained by 2×24 hour food recall and a Physical Activity Level questionnaire, respectively (FAO 2001). In addition, FBG levels were measured using the Glucose Oxidase-Peroxidase Aminoantipyrine method, while SOD levels were analyzed using the Enzyme-like Immunosorbent Assay (ELISA) method (Cayman Chemical, Ann Arbor, MI, USA).

Data analysis

Data were processed using MS Excel and SPSS 23 software for statistical analysis. The normality test was carried out using the Shapiro-Wilk test. The difference in SOD and FBG levels between groups was analyzed using one-way ANOVA, followed by Duncan's multiple range test when there was a significant result ($p<0.05$).

RESULTS AND DISCUSSION

The bioactive components of purple okra pudding and supplement

The bioactive components of purple okra pudding and supplement are presented in Table 1.

The capacity of the antioxidant compounds in purple okra extract can be expressed as Ascorbic Acid Equivalent Antioxidant Capacity

Table 1. Bioactive component of purple okra pudding and supplement

Bioactive component	Unit	Mean values	
		Supplement	Pudding
Antioxidant capacity (AEAC)	mg AEAC g^{-1}	455.39	0.39*
Antioxidant capacity (IC_{50})	ppm	71.87	543.79
Total phenol	mg GAE g^{-1}	24.49	6.21*

*1 g of purple okra pudding extract is equivalent to 50 g of purple okra pudding

(AEAC). The AEAC value of the purple okra extract supplement was 455.39 mg AEAC g^{-1}, which is different from the value obtained in previous research on purple okra extract, namely 0.04 mg AEAC g^{-1} (Anjani *et al.* 2018). This difference might be due to differences in varieties, forms of okra before extraction, and the solvents used. Meanwhile, the AEAC value of the purple okra pudding was 0.39 mg AEAC g^{-1} extract. In other words, the antioxidant capacity of 100 g of purple okra pudding is equivalent to 0.78 mg of vitamin C. The AEAC value of pudding sample found in this study is different from that obtained in previous research on boiled okra, which showed a value of 1.3 mg AEAC 100 g^{-1} (Utami 2018). With the use of 25 g of purple okra and an extra 15 g of purple okra mucilage extract, the antioxidant activity of purple okra pudding was more than half of the antioxidant activity of 100 g of boiled okra.

The antioxidant capacity that produces the % inhibition is used to obtain the IC_{50} value, which is defined as the concentration required to inhibit 50% of DPPH free radicals. Antioxidant activity of the purple okra extract supplement analyzed in this study was 71.87 ppm, which showed strong antioxidant activity (Santoso *et al.* 2022). A different value was obtained in a previous study performed on the purple okra extract, which was 316.86 ppm (Nabila *et al.* 2018). For purple okra pudding, the value of antioxidant activity (IC_{50}) found in this study was 543.79 ppm. A different value was obtained from purple okra extract analyzed in a previous study, which was 316.86 ppm (Nabila *et al.* 2018). The lower IC_{50} value in purple okra extract means that the antioxidant activity of purple okra extract was higher than that of purple okra pudding. This was due to the extraction process that resulted in a higher concentration of antioxidant compounds, leading to a higher antioxidant activity (Susanty & Bachmid 2016).

Several studies on okra have shown the presence of phenolic compounds, which are expressed as Gallic Acid Equivalents (GAE). The total phenol found in purple okra supplement used in this study was 24.49 mg GAE g^{-1}. Another study found that the strong antioxidant effect in okra sample comes from the seed extract of okra, while the peel extract shows almost no presence of these compounds. Total phenol content of okra seed extract was reported to be 56.66 mg GAE g^{-1} (Chaemsawang *et al.* 2019). Meanwhile, the pudding sample used in this study was found to have total phenol of 6.21 mg GAE g^{-1} extract. A different value was obtained from analysis of fresh purple okra in a previous study, which was 20.3 mg GAE g^{-1} (Utami 2018). The difference could occur because the previous study used fresh okra, allowing it to maintain its bioactive components, which are thermolabile (Rifkowaty & Wardanu 2016). The mechanism of antioxidant action can be due to stabilization of ROS initiators, increasing enzymatic endogenous antioxidants, and chain termination (Kurutas 2015).

Subject characteristics

A total of 30 subjects who met the inclusion criteria were included in the study. The subject characteristics are shown in Table 2.

At baseline, all groups shared the same characteristics in terms of normal nutritional status (BMI of 18.7–25.0 kg/m^2), normal FBG levels (73–99 mg/dL), but high body fat percentages (>28% for women, >20% for men). There was a significant age difference between the supplement and pudding groups due to a wider range of age of the pudding group. In adults, free radicals

Table 2. Subject characteristics before intervention period

Subject characteristics	Mean±SD			*p*
	Supplement	Pudding	Control	
Age (years)	21.70±1.90[a]	25.00±2.87[b]	23.70±2.83[ab]	0.027*
BMI (kg/m^2)	21.80±2.00	22.00±1.51	22.00±1.87	0.971
FBG (mg/dL)	87.60±7.30	84.50±5.06	85.30±7.35	0.567
BFP (%)	33.40±6.10	34.25±5.34	33.48±6.56	0.942

Statistical analysis: ANOVA followed by Duncan's multiple range test; *Significant at p<0.05

BMI: Body Mass Index; FBG: Fasting Blood Glucose; BFP: Body Fat Percentage; SD: Standard Deviation begin to accumulate from the beginning of life, which causes an imbalance in the body's oxidant and antioxidant status (Sharifi-Rad *et al.* 2020). Previous research showed different plasma MDA levels in adolescents, adults, and the elderly, with MDA values of 0.85 µM, 1.25 µM, and 2.54 µM, respectively (Mas-Bargues *et al.* 2021).

Food intake and physical activity

The data of subject's energy and nutrient intake were obtained through food intake data using a 2×24 hour food recall. Physical activity data were collected in 24 hours in the form of physical activity levels. Food intake and physical activity of the subjects are presented in Table 3.

The levels of carbohydrates, fibers, vitamin C, vitamin E, zinc adequacy, and cholesterol consumption were not significant among groups ($p>0.05$). The carbohydrate adequacy levels were categorized as moderate deficit for the supplement and pudding groups and severe deficit for the control group. All groups were in the same category for fiber, vitamin C, and vitamin E adequacy levels, namely severe deficit (Nurhidayati *et al.* 2017).

The level of energy adequacy of the pudding group was significantly higher than that of the control group but not of the supplement group. The categories of the energy adequacy level were moderate deficit for the supplement and pudding groups and severe deficit for the control group. The pudding group also demonstrated a higher protein adequacy level (mild deficit) compared to both the supplement and control groups (severe deficit). Although the levels of nutritional adequacy differed significantly, all was below 100% of the adequacy level.

Fat adequacy level of the control group was significantly lower than the pudding and supplement groups. The control group's category of fat adequacy level was normal, while the treatment groups were over the daily need (Nurhidayati *et al.* 2017). This could be due to the frequent consumption of fast food which is high in fat (Janssen *et al.* 2018). Based on the food recall analysis, the treatment groups consumed fast food 0–6 times per 8 days of food recall, while the control group only had a fast-food consumption frequency of 0–2 times. Adults prefer to consume fast food and sweetened beverages, and the trend is related to the availability and accessibility of fast food as well as hectic lifestyle (Abdullah *et al.* 2015). A study also demonstrated that 84% of university students consumed fast food (Habib *et al.* 2011). The difference in the fast food frequency consumption was likely caused by differences in the subjects' locations of residence. Groups were divided purposively to facilitate

Table 3. Food intake and physical activity of the subject

Variable	Mean±SD			*p*
	Supplement	Pudding	Control	
Level of adequacy (%)				
Energy	82.53±19.50[ab]	90.74±11.98[b]	69.82±13.94[a]	0.019*
Carbohydrate	71.54±20.45[a]	70.99±10.07[a]	61.23±14.84[a]	0.273
Protein	69.55±17.48[a]	86.64±15.20[b]	63.82±18.54[a]	0.016*
Fat	119.13±26.05[b]	141.53±27.22[b]	94.28±20.51[a]	0.001*
Fiber	21.20±9.73[a]	20.24±7.23[a]	21.88±9.31[a]	0.917
Vitamin E	15.26±4.24[a]	15.63±4.98[a]	17.74±6.01[a]	0.514
Vitamin C	29.24±31.17[a]	30.26±36.48[a]	31.84±29.85[a]	0.984
Zinc	45.68±11.52[a]	43.71±15.90[a]	51.97±16.60[a]	0.440
Cholesterol (mg)	244.22±118.55[a]	298.41±90.73[a]	196.62±61.66[a]	0.068
Physical Activity (PAL)	1.54±0.09[b]	1.36±0.06[a]	1.52±0.12[b]	<0.001*

Statistical analysis: ANOVA followed by Duncan's multiple range test; *Significant at $p<0.05$
SD: Standard Deviation

product distribution during the intervention period. The intervention groups were subjects who lived by renting a place nearby the campus with limited cooking facilities, while the control group lived at home with family members away from the campus. Thus, subjects in the control group tended to consume home-cooked food and had a lower frequency of fast food consumption than the treatment groups. High fat intake is associated with increased ROS production through the β-oxidation pathway in mitochondria (Kuchay *et al.* 2020). The accumulation of ROS production will induce lipid peroxidation, which results in MDA formation (Ayala *et al.* 2014).

The subjects' physical activity showed a significant difference in the pudding group (p<0.001), and all groups were in the same sedentary physical activity category (PAL 1.4–1.7) according to FAO (2001), with average scores of physical activity in the supplement group, pudding group, and control group being 1.54, 1.36, and 1.52, respectively.

Changes in superoxide dismutase levels

The administration of purple okra pudding and supplement for 28 days to adults with high body fat percentages had an effect on changes in the SOD levels. The SOD levels before and after intervention are shown in Table 4.

In this study, the intervention resulted in significantly different changes in SOD levels (p=0.01). The levels of quercetin as the major bioactive compound found in okra were different between supplement and pudding. The treatment group given the purple okra supplement with a quercetin content of 18.65 mg per day showed a decrease in SOD levels of 0.15±0.17 U/mL after the intervention. SOD activity was used as one of the parameters in determining the presence of antioxidant activity (Andrestian *et al.* 2019). The decrease in SOD levels of the subjects given the purple okra supplement can be explained by the role of the supplement in balancing the oxidative stress caused by free radicals. The antioxidants from the purple okra supplement are needed when endogenous antioxidant production is unable to compensate for the increase in free radicals (Moussa *et al.* 2019). The purple okra supplement has been identified as having a superior total phenol content, supported by apparent strong antioxidant activity which plays a role in changes the endogenous antioxidant levels. Flavonoids can easily donate hydrogen atoms to radical compounds, resulting in the reduction of highly oxidized radicals (Procházková *et al.* 2011; Kim *et al.* 2020).

On the other hand, there was an increase in SOD levels of 0.08±0.17 U/mL in the pudding group, which consumed 2.02 mg of quercetin per day after the intervention. This figure was significantly different from those of the supplement and control groups. The mechanism of bioactive components in functional food, such as quercetin, gives beneficial health effect by regulating the enzyme-mediated antioxidant system (Xu *et al.* 2019). The intervention of purple okra pudding, which had lower antioxidant activity (Table 1) compared to the intervention

Table 4. The changes in superoxide dismutase and fasting blood glucose levels

Group	Mean±SD			*p*
	Pre-intervention	Post-intervention	Δ	
Superoxide dismutase (U/mL)				
Supplement	0.89±0.44	0.74±0.37	-0.15±0.17[a]	
Pudding	0.67±0.10	0.75±0.09	0.08±0.17[b]	0.01*
Control	0.73±0.08	0.65±0.12	-0.07±0.15[a]	
Fasting blood glucose (mg/dL)				
Supplement	87.60±7.30	89.50±6.39	1.90±5.34	
Pudding	84.50±5.06	84.90±4.43	0.40±6.34	0.86
Control	85.30±7.35	86.50±5.58	1.20±6.53	

Data analysis: ANOVA followed by Duncan's multiple range test; *Significant at p<0.05

SD: Standard Deviation

of purple okra supplement, contributed to the increase of SOD levels, which can be explained by another endogenous antioxidant mechanism. It has been suggested that the mechanism promotes endogenous antioxidant defenses via upregulation (Kumar & Pandey 2013). The antioxidant activity and redox properties of flavonoids play a role in the activation of Antioxidant Responsive Element (ARE), which is a regulatory sequence of a group of genes encoding for phase II enzymes, including SOD (Banjarnahor & Artanti 2014). This is relevant to the fact that flavonoids such as quercetin glycosides, rutin, and isoquercitrin, which are also contained in okra, have distinct features in upregulating the production of intracellular antioxidant enzymes (Kozłowska & Szostak-Węgierek 2019). A preclinical study that examined the effects of okra pod methanol extract on lead acetate toxicity in mice kidneys concluded that the administration of okra methanol extract significantly increased SOD activity (Wahyuningsih *et al.* 2020). The two different mechanisms of purple okra supplement and pudding in changes of SOD levels are shown in Figure 1.

The control group showed a decrease in SOD levels of -0.07±0.15 U/mL, which was lower than the reduction in SOD levels of the supplement group. This indicate that the body's antioxidant defenses of the control group worked in maintaining the antioxidant status. The insignificant difference from the supplement group could be due to the variability of SOD levels, which could be influenced by other factors that were not observed in this study, such as exposure to pollutants, inflammation, physical and mental stress, radiation, etc. (Sanjay & Shukla 2021). The accumulation of free radicals from inside and outside the body that exceeds the limit of protective capacity has an impact on changes in SOD levels (Ayala *et al.* 2014).

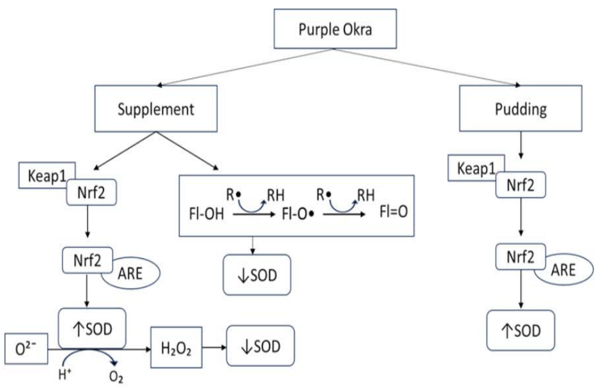

Figure 1. Hypothesized mechanism of antioxidant action from purple okra products (Modified from Xu *et al*. 2019; Kim *et al*. 2020; Liu *et al.* 2023)

The balance between oxidative and antioxidant processes appears to be sensitive to glucose levels, with early elevations of glucose affecting the oxidative status (Menon *et al.* 2004). Sujects' FBG levels were not significantly different (p=0.86), with each group having normal FBG levels. High blood glucose levels increase radical production, which affects levels of endogenous antioxidant SOD (Sachdeva *et al.* 2014). Hyperglycemia promotes collateral glucose metabolism through protein kinase C, polyol, and hexosamine routes. The mentioned processes undermine cellular structures, finally giving place to a progressively greater degree of oxidative stress with further hyperglycemia, metabolic alterations, and diabetes complications (González *et al.* 2023). FBG being at normal levels in all groups indicates that different antioxidant mechanisms play a role in maintaining normal physiological functions of the body.

CONCLUSION

Both purple okra pudding and supplement displayed potentials as sources of antioxidants based on their identified antioxidant capacity, IC_{50}, and total phenol. The intervention with both products resulted in different changes in SOD levels over the course of 28 days. This study has shown that okra intervention in different forms, pudding and supplements, possessed relatively high antioxidant activity. However, there were no significant differences between the control and treatment groups of healthy subjects. Both products can be used to maintain the balance of antioxidant status in adults. It is possible to further test the products in adults with certain health conditions to see the significant effect that they can impose.

ACKNOWLEDGEMENT

We would like to express our special thanks to the Directorate General of Higher Education, Research, and Technology of the

Ministry of Research and Technology/National Research and Innovation Body for the grant under the Downstream Applied Research Scheme 2023 (*Skema Penelitian Terapan Jalur Hilirisasi 2023*). We would also like to extend the warmest acknowledgment and thanks to the entire research team for the guidance, advice, and brilliant comments and suggestions that have led us through all the stages of this project.

DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2024, 19(1):41-50***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2024.19.1.41-50*
ISSN 1978-1059 EISSN 2407-0920

Dietary Adherence in Children with Amino Acid Metabolism Disorders and its Impact on Caregivers' Quality of Life

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ABSTRACT

This study explored the relationship among socio-demographic factors, patients' dietary adherence, and the Quality of Life (QoL) experienced by their caregivers. A cross-sectional study was carried out, involving 66 caregivers of pediatric patients in Malaysia diagnosed Amino Acid Metabolism Disorders (AAMDs). Dietary adherence was assessed using a modified version of the Malaysia Medication Adherence Assessment Tool (MyMAAT-12), and the caregiver's QoL was evaluated using the 36-Item Short Form Survey (SF 36) questionnaire. Majority of the patients were Malay (75.76%), and female (59.1%). Statistical analysis found that caregivers with a higher education level were associated with a higher dietary adherence (rs=0.382, p=0.002) and better mental health (rs=0.281, p=0.022). The age of patients had negative significant correlation with the physical function and general health (rs=0.287, p=0.019) of their caregivers. Besides that, dietary adherence was negatively correlated with social functioning (p=0.010), role limited due to physical health (p=0.018), role limited due to emotional problems (p=0.022), vitality (p=0.021), mental health (p=0.014) and pain (p=0.011). Conclusion: Dietary adherence had a significant impact on the QoL for caregivers of patients with amino acid metabolism disorders. Therefore, it is crucial to explore appropriate treatment strategies and provide support to enhance patients' adherence to their dietary requirements, simultaneously improving the caregivers' QoL.

Keywords: amino acid metabolism, caregivers, inborn errors, mental health, quality of life

INTRODUCTION

Amino Acid Metabolism Disorders (AAMDs) include a spectrum of conditions, such as Organic Acidaemias (OA) including Methylmalonic (MMA), Propionic (PA), Isovaleric Acidaemias (IVA), Glutaric Aciduria (GAT1), and others; aminoacidopathies such as Maple Syrup Urine Disease (MSUD), Phenylketonuria (PKU), Homocystinuria (HCS), and Tyrosinemia (TYS) type 1; as well as Urea Cycle Disorders (UCD), which are rare Inherited Metabolic Disorders (IMD) that occur due to a defect in amino acid catabolism (Saudubray *et al.* 2013; Ezgu 2016). Most of these conditions are inherited autosomal recessive traits, with the exception of Ornithine Transcarbamylase (OTC) deficiency, which follows an x-linked genetic disorder (De Meirleir & Rodan 2017). These disorders lead in the accumulation of toxic intermediary metabolites and deficiencies in various essential metabolites (Boyer *et al.* 2015).

Generally, individuals with amino acid metabolism disorders require an individualised dietary treatment that includes stringent limitations on natural protein intake and supplementation

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(Received 14-08-2023; Revised 31-10-2023; Accepted 01-03-2024; Published 30-03-2024)

with synthetic amino acid formulas. The main goal of dietary therapy is to reduce the accumulation of toxic substances, while ensuring an adequate supply of energy, protein, vitamins, and minerals for optimal growth and development (Boyer *et al.* 2015; Dixon *et al.* 2014).

However, the strict adherence of lifelong dietary and medical treatment for pediatric patients with AAMDs imposes a considerable burden for caregivers, requiring exclusive dedication that could also potentially compromise their well-being (Lim *et al.* 2022; Zeltner *et al.* 2019; Martianto *et al.* 2011). Research demonstrates that adhering to these specialized diets can contribute to higher perceived stress and a lower Quality of Life (QoL) among the caregivers (Fabre *et al.* 2013). Parents reported notable burdens and challenges, including in the preparation of meals with specific weighing requirements, time constraints, and restrictions on social life (Bilginsoy *et al.* 2005; Fabre *et al.* 2013). These complications have been linked to increased mental, emotional, and interpersonal stress among caregivers (Yamaguchi *et al.* 2018). In fact, several studies have indicated that dietary management is the leading cause of caregivers' poor QoL (Rohde *et al.* 2015; Ford *et al.* 2018). Other relevant factors associated with caregivers' QoLs (Fidika *et al.* 2013) include the child's age, household income, educational level (Tejada-Ortigosa *et al.* 2019), and parental stress (Morawska *et al.* 2020) .

Specifically, the decline in caregivers' QoL is primarily linked to dietary supervision, particularly when caring for patients with restrictive diets due to amino acid metabolism disorders (Carpenter *et al.* 2018). However, to the best of the author's knowledge, there is a lack of research investigating the relationship between dietary adherence and the QoL of caregivers for patients with disorders of amino acid metabolism in Malaysia. As such, this present study aimed to address this literature gap by examining the relationships between socio-demographic factors, dietary adherence of pediatric patients with these disorders, and the QoL of their caregivers in the Malaysian context.

METHODS

Design, location, and time

This study employed a cross-sectional design to examine the relationship between socio-demographic factors, the dietary adherence of pediatric patients with AAMDs, and the QoL of their caregivers. A total of 66 eligible caregivers of affected patients participated in the study, which was conducted between October and December 2020.

This study received approval and registration from both the National Medical Research Register (NMRR-20-138-52694) and the Research Ethics Committee UKM (JEPUKM-615 2021-765). Informed consent was obtained from the parents or legal guardians of participating patients, as well as from caregivers.

Sampling

Purposive sampling was used, for which the inclusion criteria were Malaysian citizen, patients aged under 18 years with disorders of amino acid metabolism their caregivers aged above 18 years who were receving treatment at the Genetic Clinic in Hospital Kuala Lumpur, the national referral centre for the treatment of patients with genetic diseases.

Using a combined sampling strategy, all eligible caregivers of patients with AAMDs attending the Genetic Clinic at Hospital Kuala Lumpur during the period October-December 2020 were invited to participate. This approach was necessitated by the limitations in readily available data regarding the precise population size of AAMD patients in Malaysia, which estimated to range from 74 to over 100 patients (Shafie *et al.* 2020).

Study measurement. Data collection included questionnaires evaluating participants' socio-demographic details, dietary adherence, and the caregivers' QoL. The questionnaires underwent a meticulous back-translation process to ensure their cultural and linguistic equivalence. Initially translated from English to Malay by two experienced bilingual researchers, the questionnaires were then subsequently retranslated back into English. These translated versions were presented at a research meeting to identify and address any potential ambiguities or discrepancies in wording, sentence structure, and intended meaning. Following reviews and adjustments, as well as unanimous consensus by all research team members, the socio-demographic questionnaire were finalized. It comprehensively assessed subject-specific information, such as gender, age, ethnicity, education level, occupational status, marital status, parental consanguinity, monthly household income, and other pertinent details. Dietary adherence was assessed using the adapted Malaysia Diet Adherence Assessment Tool (MyDAAT-12). This tool, derived from the MyMAAT-12, was constructed by the Faculty of Pharmacy UKM and the Ministry of Health Malaysia (Hatah *et al.* 2020). My DAAT-12 scores reflect the likelihood of patients adhering to their prescribed diets, with higher scores indicating greater adherence and vice versa. The tool utilizes a 5-point Likert scale (1=Strongly disagree; 2=Disagree; 3=Meutral; 4=Agree; 5=Strongly agree). A total score of 54 (90%) and above indicated good adherence, reflecting its clinical importance of strict adherence due to the nature of the disorder. Scores below 54 were classified as poor adherence. The modified questionnaire exhibited robust internal consistency reliability, with a Cronbach's alpha of 0.826.

The caregivers' QoL was assessed using the widely utilized 36-Item Short Form Survey (SF-36) questionnaire. The questionnaire is a preferred measure for evaluating Health Related Quality of Life (HrQoL) among IEM's patients (Pascoal *et al.* 2018). It includes eight domains: physical functioning, physical role, pain, general health, vitality, social function, emotional role, and mental health. Higher scores on each domain indicate netter QoL.

Data collection

Following the provision of a detailed information sheet outlining the study's objectives, benefits, risks, and data confidentiality measures, participants provided informed consent and proceeded to complete self-administered questionnaires. The questionnaires, requiring an estimated 30–60 minutes to complete, were designed to collect primary data on participants' socio-demographics, dietary adherence, and quality of life.

Data analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., 2015). Descriptive statistics were employed to characterize the socio-demographic profiles of both patients and caregivers. Normally distributed continuous variables were presented as mean and standard deviation, while skewed data was reported as median and interquartile range. Categorical variables were summarized by frequency (n) and percentage (%). Spearman's rank correlation coefficient was used to determine the potential associations between socio-demographic variables, dietary adherence, and the QoL of the caregivers. Statistical significance was established if $p<0.05$.

RESULTS AND DISCUSSION

The study included 66 caregivers, categorized based on the underlying disorders of the patients they cared for. Among these, 36.4% (n=24) were caregivers of patients with OA, including Propionic Acidemia (PA; n=9), Glucose Transporter Type 1 deficiency (GLUT1; n=7), Methylmalonic Acidemia (MMA; n=4), Isovaleric Acidemia (IVA; n=2), and Hydroxymethylglutaryl-CoA (HMG-CoA) lyase deficiency (n=2). The remaining participants cared for patients with either aminoacidopathies (37.9%, n=25) – encompassing Maple Syrup Urine Disease (MSUD; n = 18), Phenylketonuria (PKU; n=3), Tyrosinemia Type 1 (TYS; n=3), and Homocystinuria (HCS; n=1) – or Urea Cycle Disorders (UCD) (25.8%; n=17). The mean ages of patients with OA, aminoacidopathies, and UCD were 6.42±4.75, 9.46±4.41 and 8.38±4.96 years, respectively. An analysis of patient ethnicity revealed a majority of Malay individuals across all groups: 58.3% (OA), 76% (aminoacidopathies), and 100% (UCD). This finding aligns with prior research conducted in the Malaysian context (Pei *et al.* 2014; Thong & Yunus 2008). However, further research is warranted to investigate the potential reasons for this observed higher prevalence of amino acid metabolism disorders among the Malay population. Regarding education, the majority of OA patients (62.5%) did not attend school, while 16.7% received special education.

Parental consanguinity rates varied across the groups, observed in approximately one-fifth of OA (20.8%) and UCD (23.5%) cases, and exceeding one-third (32.0%) in MSUD cases (Table 1). There findings align with previous studies reporting a higher prevalence of parental consanguinity among patients with IMDs (Kong *et al.* 2014; Thong & Yunus 2008; Najafi *et al.* 2016). A prior cross-sectional study among Malaysians with IMDs found a parental consanguinity rate of 40.9% in MSUD patients (Kong & Roslee 2015). Consistent with these findings, the present study observed a relatively high prevalence of parental consanguinity in patients with AAMDs, with about one-third of taminoacidopathies and one-fifth of OA and UCD patients exhibiting this characteristic. An Iranian study reported a prevalence of parental consanguinity at 84.7% among OA patients (Najafi *et al.* 2016). Notably, in this study, the majority of the caregivers were mothers, a trend consistent with (Khangura *et al.* 2016).

It was found that most caregivers in the most possessed a secondary school education (54.55%), followed by individuals who graduated from college or university (36.36%). Majority of the caregivers were married (OA: 83.3%, aminoacidopathies: 88.0%, UCD: 94.1%). Regarding employment, most OA caregivers were full-time housewives (66.7%), followed by working people (29.2%), and retirees (4.2%). In contrast, a higher portion of caregivers in the aminoacidopathies (64.0%) and UCD (52.9%) cohorts were employed (Table 2). In fact, more than half of the caregivers for patients with aminoacidopathies and UCD in this study were still working, contrasting with findings from studies in Spain and the UK. Tejada-Ortigosa *et al.* (2019) reported that 77.4% of the caregivers in Spain needed to reduce their working hours or quit their jobs to care for their children with IMD.

Table 1. Sociodemographic profile of the patients with disorders of amino acid metabolism (n=66)

Variables	OA (n=24)	Aminoacidopathy (n=25)	UCD (n=17)
Age of patients, years, mean±SD	6.42 (4.75)	9.46 (4.41)	8.38 (4.96)
Gender, n (%)			
Male	13 (54.2)	11 (44.0)	3 (17.6)
Female	11 (45.8)	14 (56.0)	14 (82.4)
Ethnicity, n (%)			
Malay	14 (58.3)	19 (76.0)	17 (100)
Chinese	6 (25.0)	3 (12.0)	-
Indian	1 (4.2)	1 (4.0)	-
Others	3 (12.5)	2 (8.0)	-
Stage of onset, n (%)			
Early onset (≤28 days)	11 (45.8)	13 (52.0)	7 (41.2)
Late onset (>28 days)	13 (54.2)	12 (48.0)	10 (58.8)
Education level for patients, n (%)			
Preschool	-	2 (8.0)	1 (5.9)
Kindergarten	-	4 (16.0)	1 (5.9)
Primary school	4 (16.7)	7 (28.0)	2 (11.8)
Secondary school	1 (4.2)	3 (12.0)	2 (11.8)
Special education school	4 (16.7)	5 (20.0)	5 (29.4)
Informal education	1 (4.2)	-	-
Not schooling	14 (58.3)	4 (16.0)	6 (35.3)
Type of treatment			
Diet and medical treatment	24 (100)	25(100)	17(100)
Family history, n (%)			
Yes	1 (4.2)	3 (12.0)	2 (11.8)
No	20 (83.3)	18 (72.0)	14 (82.4)
Does not know	3 (12.5)	4 (16.0)	1 (5.9)
Parental consanguinity, n (%)			
Yes	5 (20.8)	8 (32.0)	4 (23.5)
No	16 (66.7)	16 (64.0)	13 (76.5)
Does not know	3 (12.5)	1 (4.0)	-

OA: Organic Acidaemias; SD: Standard Deviation; UCD: Urea Cycle Disorders

More than half of the OA patients in this study did not attend school or received special education, aligning with findings from a cross-sectional study by Najafi *et al.* (2016), where only 9.7% of OA patients were enrolled in mainstream education. This pattern may be attributed to the frequent association of OA with both convulsions and developmental disorders (Thomas *et al.* 2017). The substantial proportion of OA patients not attending school potentially contributes to the observation that most OA caregivers were full-time housewives (66.7%), facilitating childcare at home. This aligns with reports indicating that one-third of OA patients may experience developmental delays, potentially requiring additional caregiving support (Najafi *et al.* 2016).

Among the participants, 40 patients (60.61%) displayed good adherence to their prescribed diet (Table 3). This adherence was primarily motivated by the patients' desire to prevent neurological problems and metabolic crises, while balancing the need for sufficientenergy and protein intake. These findings align with previous study by Bilginsoy *et al.* (2005), who reported that 72% of the PKU families in Utah maintained adequate energy consumption, indicative of successful dietary management. A UK cross-sectional study involving 106 PKU patients reported only 8% with poorly controlled blood phenylalanine status, indicating that most of the patients had good adherence to a low-natural-protein diet

Table 2. Sociodemographic profile of the caregivers of the patients with disorders of amino acid metabolism (n=66)

Variables	OA (n=24)	Aminoacidopathy (n=25)	UCD (n=17)
Age of caregivers, years (Mean±SD)	37.08±11.83	39.32±8.31	40.00±7.17
Relationship, n (%)			
Mother	21 (87.5)	20 (80.0)	12 (70.6)
Father	1 (4.2)	4 (16.0)	5 (29.4)
Grandparents	1 (4.2)	-	-
Others	1 (4.2)	1 (4.0)	-
Ethnicity, n (%)			
Malay	14 (58.3)	19 (76.0)	17 (100)
Chinese	6 (25.0)	3 (12.0)	-
Indian	1 (4.2)	1 (4.0)	-
Others	3 (12.5)	2 (8.0)	-
Education level for caregivers, n (%)			
Does not go to school	1 (4.2)	1 (4.0)	-
Primary school	3 (12.5)	-	1 (5.9)
Secondary school	13 (54.2)	15 (60.0)	8 (47.1)
Diploma/Degree (Bachelor/Master/PHD)	7 (29.2)	9 (36.0)	8 (47.1)
Marital status, n (%)			
Single	1 (4.2)	3 (12.0)	1 (5.9)
Married	20 (83.3)	22 (88.0)	16 (94.1)
Divorced	3 (12.5)	-	-
Number of children dependant	2.4 (1.2)	3.3 (1.5)	2.9 (1.9)
Range	1 to 5	1 to 6	1 to 6
Employment status, n (%)			
Working	7 (29.2)	16 (64.0)	9 (52.9)
Retirees	1 (4.2)	-	-
Retired but still working	-	-	-
Unemployed/housewife	16 (66.7)	9 (36.0)	8 (47.1)
Monthly household salary (Mean±SD) (MYR)	4,366.67 (3,856.77)	6,228.0 (7,334.59)	5,135.29 (5,197.35)
Monthly food budget (Mean±SD) (MYR)	1,293.75 (1,229.73)	1,413.40 (1,110.76)	1,485.29 (1,089.12)

MYR: Malaysian Ringgit; OA: Organic Acidaemias; SD: Standard Deviation; UCD: Urea Cycle Disorders

Table 3. Dietary adherence of the patients with disorders of amino acid metabolism (n=66)

Dietary adherence	OA (n=24)	Aminoacidopathy (n=25)	UCD (n=17)	Total
Good adherence, n (%)	14 (58.3)	14 (56.0)	12 (70.6)	40 (60.6%)
Poor adherence, n (%)	10 (41.7)	11 (44.0)	5 (29.4)	22 (33.3%)

OA: Organic Acidaemias; UCD: Urea Cycle Disorders

(MacDonald *et al.* 2016). This contrasts with a Brazilian cross-sectional study of 56 PKU patients, where only 18 (32.1%) were classified as treatment adherent (Vieira *et al.* 2018). Notably, no significant differences in any of the QoL domains were observed between groups (Table 4).

A statistically significant, albeit weak positive correlation was found between caregiver education level o and dietary adherence (rs (64)=0.382, p=0.002). This finding suggests a potential association between higher caregiver education levels and improved dietary adherence among pediatric patients.

Table 5 shows the correlations between socio-demographic variables, dietary adherence, and quality of life domains. Caregiver education level oexhibited a significant positive correlation with mental health (rs (64)=0.281, p=0.022) (Table 5). However, dietary adherence displayed a weak negative correlation with various QoL domains, including role limitations due to physical health (rs (64)=-0.290, p<0.05), role limitations due to emotional problems (rs (64)=-0.282, p<0.05), bodily pain (rs (64)=-0.309, p<0.05), vitality (rs (64)=-0.284, p<0.05), mental health (rs (64)=-0.300, p<0.05) and social functioning (rs (64)=-0.314, p<0.05). Dietary adherence was found to negatively correlated with all QoL domains.

In the present study, association between higher caregiver education levels and improved dietary adherence, reflecting enhanced knowledge of the treatment regimen (Tejada-Ortigosa *et al.* 2019). This finding aligns with previous studies demonstrating the link between lower education level attainment and poorer knowledge of the treatment, leading to lower dietary adherence (Adam *et al.* 2013; Öztürk *et al.* 2022). However, our findings diverge from a previous study by Zeltner *et al.* (2019), which reported a decline in dietary compliance with advancing age, particularly around 10 years old.

Caregivers of female patients reported lower role limitations due to emotional problems. It was found that Malay caregivers had lower scores in various domains, including role limitations (physical and emotional), bodily pain, vitality, and mental health. However, the generalizability of these findings may be limited due to the high proportion of Malay participants (over two-thirds) within the sample. Further studies are needed to explore the associations between gender, ethnicity, and QoL while considering diverse demographic representation. Additionally, our findings suggest an association between higher caregiver education and better mental health. This could be attributed to enhanced

Table 4. Quality of life of caregivers of the patients with disorders of amino acid metabolism (n=66)

Categories of disorders of amino acid metabolism	Physical function	Role limitations due to physical health	Role limitations due to emotional problems	Bodily pain	General health	Vitality	Mental health	Social functioning
	Median (IQR)							
OA	80.00 70.00–95.00	75.00 25.00–100.00	100.00 33.00–100.00	88.75 57.50–100.00	72.50 60.00–80.00	65.00 55.00–75.00	68.00 56.00–84.00	81.25 62.50–100.00
Aminoacidopathy	85.00 70.00–90.00	75.00 25.00–100.00	100.00 50.00–100.00	77.50 51.25–100.00	70.00 62.50–80.00	65.00 50.00–77.50	68.00 50.00–84.00	75.00 62.50–87.50
UCD	90.00 77.50–95.00	100.00 87.50–100.00	100.00 83.33–100.00	80.00 56.25–90.00	80.00 47.50–95.00	70.00 52.40–80.00	80.00 68.00–90.00	75.00 62.50–93.75
p	0.287	0.135	0.454	0.670	0.811	0.785	0.215	0.405

IQR: Interquartile Range; OA: Organic Acidaemias; SD: Standard Deviation; UCD: Urea Cycle Disorders; p-value tested by using Kruskal-Wallis test at p<0.05

knowledge of treatment regimens, leading to improved patient management and ultimately, caregiver well-being. It is important to note that the link between low education and depression (Niemeyer *et al.* 2019) highlights the potential for broader mental health considerations among caregivers with lower education attainment.

This study identified a negative correlation between patients' age and caregivers' QoL, aligning with findings from a German cross-sectional study of 89 PKU parents (Fidika *et al.* 2013). The study found that parents with children under six years old had significantly lower mean scores for "self-development" compared to parents of older children. Younger children, being unable to independently manage their own diets, required increased parental support and supervision. Hence, it can be inferred that caregivers experienced a higher QoL due to the greater parental freedom available when their child attended school from the age of six and above (Fidika *et al.* 2013). Contrary to our current findings, parents with higher monthly income and younger age showed higher quality of life (Thomas *et al.* 2017). Nevertheless, this discrepancy might be attributed to the fact that medical visits and medical food are fully reimbursed by the Ministry of Health in Malaysia.

Table 5. Correlation of socio-demographic parameter and dietary adherence with quality of life

Variables	Physical function	Role limitations due to physical health	Role limitations due to emotional problems	Bodily pain	General health	Vitality	Mental health	Social functioning
	rs, Sig.	rs, Sig.	rs, Sig.	rs, Sig.	rs, Sig.	rs, Sig.	rs, Sig.	rs, Sig.
Type of IEMs	-0.027, 0.829	-0.014, 0.910	-0.205, 0.099	-0.216, 0.082	-0.027, 0.829	-0.160, 0.198	-0.240, 0.052	-0.149, 0.232
Age of patients	-0.287*, 0.019	-0.039, 0.757	-0.004, 0.976	-0.110, 0.379	-0.287*, 0.019	-0.238, 0.054	-0.205, 0.098	-0.099, 0.431
Gender	-0.048, 0.702	-0.240, 0.053	-0.277*, 0.024	-0.231, 0.062	-0.048, 0.702	-0.075, 0.551	-0.166, 0.184	-0.139, 0.267
Ethnicity	-0.181, 0.146	-0.286*, 0.020	-0.269*, 0.029	-0.254*, 0.040	-0.181, 0.146	-0.261*, 0.034	-0.252*, 0.041	-0.202, 0.103
Stage of onset	0.016, 0.898	0.016, 0.200	0.083, 0.506	-0.077, 0.536	-0.006, 0.959	-0.077, 0.536	0.126, 0.313	0.242, 0.051
Education level of patients	0.134, 0.284	0.056, 0.654	0.041, 0.743	0.120, 0.338	0.134, 0.284	0.059, 0.637	0.081, 0.518	0.206, 0.097
Age of caregivers	-0.120, 0.336	-0.082, 0.514	-0.033, 0.793	-0.091, 0.468	-0.120, 0.336	-0.174, 0.162	-0.053, 0.675	0.044, 0.724
Relationship	0.144, 0.247	-0.067, 0.591	-0.152, 0.222	-0.101, 0.421	0.144, 0.247	-0.105, 0.400	0.017, 0.893	-0.131, 0.296
Number of children	-0.098, 0.433	-0.149, 0.232	0.070, 0.577	-0.094, 0.453	-0.098, 0.433	-0.103, 0.411	-0.065, 0.605	0.033, 0.793
Marital status	-0.177, 0.156	-0.015, 0.904	-0.040, 0.750	0.097, 0.441	-0.177, 0.156	-0.027, 0.831	0.012, 0.926	0.092, 0.464
Educational level of caregiver	0.178, 0.152	0.229, 0.064	0.161, 0.197	-0.094, 0.452	0.178, 0.152	0.001, 0.991	0.281*, 0.022	0.086, 0.493
Working status	-0.186, 0.135	-0.078, 0.536	-0.019, 0.881	0.006, 0.963	-0.186, 0.135	-0.040, 0.749	-0.018, 0.889	0.003, 0.979
Household salary	0.009, 0.945	0.091, 0.465	0.170, 0.173	0.111, 0.374	0.009, 0.945	-0.041, 0.746	-0.213, 0.086	0.101, 0.420
Food budget	-0.070, 0.574	0.083, 0.510	0.137, 0.271	0.013, 0.918	-0.070, 0.574	0.073, 0.560	-0.006, 0.962	0.023, 0.857
Dietary adherence	-0.178, 0.153	-0.290*, 0.018	-0.282*, 0.022	-0.309*, 0.011	-0.183, 0.141	-0.284*, 0.021	-0.300*, 0.014	-0.314*, 0.010

rs: Spearman correlation coefficient ; Correlation (*) is significant at $^{0.05}$ level (^{2} tailed)

Among the barriers to dietary adherence are burden of dietary treatment, diet and dietary behavior, parenting challenges, limited knowledge related to dietary treatment, and challenges in healthcare system delivery (Lim *et al.* 2022). Our study supports the hypothesis that increase in dietary adherence leads to a lower QoL. This aligns with findings from MacDonald *et al.* (2016), who reported that caregivers managing PKU in the UK experienced time-related stress. This stress emanated from the constant need for caregivers to engage with other families and nursery teachers, ensuring ongoing communication, supervision, and maintenance of appropriate dietary management. Furthermore, caregivers of children with PKU identified meal planning, reparation, and cooking of low-phenylalanine meals as the most time-consuming aspects of dietary management. This burden was further amplified in social settings due to the limited availability of suitable low-protein options, necessitating additional meal preparation efforts. Bilginsoy *et al.* (2005) reported that these factors, along with the stress of record-keeping and social life limitations, were the primary obstacles to dietary adherence. Both caregivers and patients expressed frustration with the dietary restrictions imposed by PKU, particularlythe inability to share certain foods (Fabre *et al.* 2013). Morawska *et al.* (2020) identified several factors contributing to a lower QoL among caregivers, includin parental guilt, the financial burden of a low-protein diet and supplements, as well as the emotional stress caused by their child's anxiety during blood tests. Additionally, the demands of preparing specialized meals significantly impact the daily lives of caregivers, reducing their opportunities for social interaction (Thomas *et al.* 2017).

This study faced the challenge of a limited sample size due to the COVID-19 pandemic, which restricted travel for patients from outside of Kuala Lumpur, especially those residing on the West Coast of Malaysia. Despite this limitation, the study holds significant value. This is the first study in Malaysia to examine the differences between the socio-demographic profiles, dietary adherence levels, and QoL across three distinct categories of pediatric patients with AAMDs and their caregivers. This broader scope, encompassing various conditions beyond the commonly studied PKU or MSUD, offers valuable insights into the diverse experiences and potential needs of this population within the Malaysian context.

CONCLUSION

This study revealed a correlation between dietary adherence in pediatric patients with AAMDs and their caregivers' QoL. Specifically, greater dietary adherence was associated with lower QoL among the caregivers, likely due to the increased time burden associated with managing the low-protein diet, including meal planning and preparation. Socio-demographic factors also played a role. Caregivers with higher levels of education showed a positive association with increased dietary adherence in their children and their own mental health.

Consequently, further research efforts should prioritize a qualitative study to gain a comprehensive understanding of the specific challenges impacting caregivers' QoL within this context. Identifying these challenges can reveal previously unidentified factors affecting their well-being.

ACKNOWLEDGEMENT

This project was funded by the Ministry of Higher Education Malaysia Fundamental Research Grant Scheme (FRGS/1/2020/SS0/UKM/02/14). The authors confirm independence from the sponsors; the sponsors have not influenced content of the article.

DECLARATION OF CONFLICT OF INTERESTS

All the authors declared that they have no conflict of interest.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan **J. Gizi Pangan, March 2024, 19(1):51-60**
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2023.19.1.51-60*
ISSN 1978-1059 EISSN 2407-0920

Influencing Factors for Malnutrition in Chronic Kidney Disease Patients: A Systematic Review and Meta-Analysis

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ABSTRACT

This study reviewed the evidence on influencing factors for malnutrition in Chronic Kidney Disease (CKD) patients. A systematic search of PubMed, Web of Science, EMBASE, Cochrane Library, China National Knowledge Infrastructure, and Wanfang Data Knowledge Service Platform for English and Chinese language observational studies published from 1966 to 2022 was conducted. RevMan 5.4.1 software was used for statistical analysis. A total of 13 observational studies with 7,790 study participants were included in the systematic review and meta-analysis. Seven risk factors and two protective factors related to malnutrition were identified. Age (OR=1.29; 95% CI:1.03–1.61), required feeding assistance (OR=3.33: 95% CI:2.55–4.35), living status (with family) (OR=0.49; 95% CI:0.34–0.71), protein intake (OR=0.89; 95% CI:0.85–0.94), comorbidities (OR=1.78; 95% CI:1.03–3.07), long dialysis duration (OR=1.61; 95% CI:1.16–2.24), inadequate dialysis (OR=1.25; 95% CI:1.12–1.40), hemoglobin level (OR=1.84; 95% CI:0.92–3.66), and depression (OR=3.44; 95% CI:2.21–5.34) were associated with an increased influence of malnutrition among CKD patients. This review provides comprehensive evidence of potential influencing factors of malnutrition among CKD patients.

Keywords: chronic kidney disease, influence factors, malnutrition, meta-analysis, systematic review

INTRODUCTION

Chronic Kidney Disease (CKD) is a general term used for heterogeneous disorders that affect renal structure and function. At present, malnutrition has emerged as a prevalent clinical complication among CKD patients. Malnutrition is an imbalance between nutrient intake and requirement, leading to deficits in energy, protein, or micronutrients that can adversely impact growth, development, and other critical outcomes (Becker *et al.* 2014). The prevalence of malnutrition among CKD patients in stages 3 to 5 is estimated to range from 11% to 54% worldwide (Carrero *et al.* 2018).

Malnutrition is a life-threatening problem for CKD patients, because has been linked to various adverse health outcomes, heightening the risk of morbidity, mortality, and overall disease burden among CKD patients. It is also an important risk factor for the malignant progression of CKD (Iorember 2018).

Due to the exact pathogenesis of malnutrition in CKD patients is complex, malnutrition often goes undetected and untreated (Barril *et al.* 2022). Therefore, identification of potentially modifiable influencing factors on malnutrition is critical for early implementation of effective interventions. However, in previous studies, the influencing factors for malnutrition are varied, and sometimes the results were inconsistent (Windahl *et al.* 2018; Omari *et al.* 2019; Namuyimbwa *et al.* 2018). In this light, we have conducted a systematic review and meta-analysis to assess malnutrition factors among CKD patients. This aims to identify these factors

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(Received 29-08-2023; Revised 15-11-2023; Accepted 11-12-2023; Published 30-03-2024)

early, allowing us to take protective measures to reduce malnutrition occurrence, thereby improving CKD patients' health.

METHODS

Design, location, and time

The design of this study was a systematic review on published articles from 1966 to 2022, using Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines throughout all the processes (PRISMA 2020). The review protocol was registered in PROSPERO 2022 (CRD42022353103). The inclusion criteria of this review includes: 1) peer-reviewed observational studies written in English and Chinese; 2) the study population age was 18 and above, diagnosed with CKD; 3) focusing on influencing factors for malnutrition, and 4), used valid and reliable parameters or instruments to evaluate malnutrition. While the exclusion criteria were includes:1) incomplete study results or have only single-factor analysis results reported; and 2) literature with a "low" quality rating (a score of < four corresponds to low quality).

Data collection

Data sources and search strategies. Six electronic databases, namely, PubMed, Web of Science, EMBASE, Cochrane Library, China National Knowledge Infrastructure, and Wanfang Data Knowledge Service Platform, were used to search for related articles.

Data extraction and quality appraisal. The EndNote X9 reference manager software was utilized for collecting and organizing search results and eliminating duplicate articles. Initial screening of study titles was conducted independently by two qualified reviewers based on accessibility criteria, followed by abstract screening. Subsequently, a re-evaluation was performed to determine the inclusion of studies in the meta-analysis, considering the full text. Discrepancies were resolved via discussions involving a third reviewer, facilitating consensus (Rimbawan *et al.* 2022).

The Newcastle–Ottawa Scale (NOS) (Stang 2010) was used to evaluate the quality of the case-control and cohort studies. Cross-sectional studies were conducted using Joanna Briggs Institute (JBI) Tool (Munn *et al.* 2015) to assess quality. For NOS and JBI scores, a score <four is considered low quality, four to six is medium quality, and ≥seven is high quality. Studies were included with scores ≥four.

Data analysis

Statistical analysis was conducted employing RevMan 5.4.1 software. To gauge the heterogeneity among the included studies, the I^2 statistics was employed. When $p \geq 0.1$ and $I^2 \leq 50\%$, indicative of homogeneity among studies, a fixed-effects model was used for description. Conversely, a random-effects model was employed for depiction in cases of substantial inter-study heterogeneity. Sensitivity analysis was conducted to evaluate the influence of individual studies on the overall effect estimation. The significance level was set at $\alpha=0.05$.

RESULTS AND DISCUSSION

A total of 5,462 articles were searched, and 4,845 articles remained after eliminating duplicates. After screening by title and abstract, 277 articles remained. The full text was read to exclude 264 articles, and 13 articles were finally included. The screening process is shown in Figure 1. In total, the combined patient sample encompassed 7,790 individuals, with samples

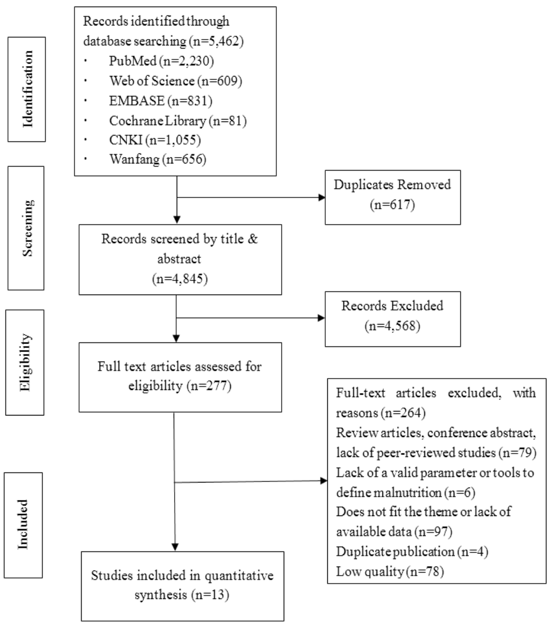

Figure 1. PRISMA flow chart of study selection process

size ranging from 53 to 2,151 patients. The detailed characteristics are summarized in Table 1.

The 10 possible influencing factors related to malnutrition were analyzed. Age was estimated as a risk factor for malnutrition among CKD patients based on five studies (Namuyimbwa *et al.* 2018; Omari *et al.* 2019; Wang *et al.* 2016; Windahl *et al.* 2018; Hwang *et al.* 2009). The pooled data under random model showed an OR of 1.29 (95% CI:1.03–1.61; p=0.02), with substantial heterogeneity (I^2=87%, p<0.01) (Table 2). Forest plot of age is presented in Figure 2 (A).

Investigators in two studies reported on relationships between the required feeding assistance and malnutrition among CKD patients (Boaz *et al.* 2019; Zhu *et al.* 2020).With an OR of 3.33 (95% CI:2.55–4.35; p<0.01), the required feeding assistance showed a significant trend towards higher malnutrition risk, under fixed-effect model (I^2=0, p=0.70) (Table 2). Forest plot of required feeding assistance is shown in Figure 2 (B).

Living status (with family) (I^2=0, p=0.49) was regarded as a protective factor associated with malnutrition, based on two included studies (Omari *et al.* 2019; Zhu *et al.* 2020). Analysis was conducted by fixed-effect model, with an OR of 0.49 (95% CI:0.34–0.71; p<0.01), presenting statistical significance (Table 2). Forest plot of living status (with family) is displayed in Figure 2 (C).

Data on CKD patients with protein intake were available from three studies with a pooled OR of 0.89 (95% CI:0.85–0.94; p<0.01) from the fixed-effect model with I^2=0 (p=0.97) (Wang *et al.* 2012; Windahl *et al.* 2018; Dahl *et al.* 2022) (Table 2). Meta-analysis results showed that higher protein intake as a protective factor against malnutrition in CKD patients. Forest plot of protein intake is shown in Figure 2 (D).

Two studies which examining the impact of comorbidities on the nutritional conditions among CKD patients were pooled in the analysis (Omari *et al.* 2019; Wang *et al.* 2012), revealing comorbidities as significance risk factors for CKD (OR=1.78; 95% CI:1.03–3.07; p=0.04; fixed-effect model). No between-study heterogeneity was found, with I^2=0 (p=0.44) (Table 2). Forest plot of this analysis on comorbidities is presented in Figure 2 (E).

Based on data from three research (Omari *et al.* 2019; Wang *et al.* 2016; Zhu *et al.* 2020), CKD patients with lengthy dialysis duration had a substantial relationship with malnutrition. The OR was 1.61 (95% CI:1.16–2.24; p=0.01, fixed-effect model), with no between-study heterogeneity (I^2=0, p=0.64) (Table 2). Forest plot of long dialysis duration is illustrated in Figure 2 (F).

Inadequate dialysis (Li *et al.* 2018; Wang *et al.* 2016; Wang *et al.* 2012) was estimated as a risk factor for malnutrition based on three studies, yielding an OR of 1.25 (95% CI:1.12–1.40; p<0.01, random model). Statistical heterogeneity was high between studies (I^2=60%, p=0.08) (Table 2). Forest plot of inadequate dialysis is presented in Figure 2 (G).

ALB, as evaluated in three studies, was identified as a potential risk factor for malnutrition in CKD patients (Guligowska *et al.* 2020; Li *et al.* 2018; Wang *et al.* 2016). In the overall pooled estimate with random model, the ALB factor played a statistically significant negative role (OR=0.70; 95% CI:0.44–1.10; p=0.13) with severe between study heterogeneity (I^2=94%, p<0.01) (Table 2). Forest plot of ALB is shown in Figure 2 (H).

Hb was significantly associated with malnutrition among CKD patients, based on two studies (Boaz *et al.* 2019; Namuyimbwa *et al.* 2018). The OR was 1.84 (95%:0.92–3.66; p=0.08, random model), with high between study heterogeneity (I^2=62%, p=0.11) (Table 2). Forest plot of Hb is presented in Figure 2 (I).

Presence of a depression significantly correlated with malnutrition in CKD patients (OR=3.44; 95% CI:2.21–5.34; p=0.08) under fixed-effect model in all three selected studies (Boaz *et al.* 2019; Czira *et al.* 2011; Namuyimbwa *et al.* 2018), with no between-study heterogeneity of I^2=0 (p=0.55) (Table 2). Forest plot of depression is presented in Figure 2 (J).

The fixed-effects model and the random-effects model were employed to combine the effects sizes separately. The values of the combined effects of the two models are close to each other, which indicates that the results are more reliable. However, the results of the two models of ALB are inconsistent (Table 3). The fixed effects model results in OR=0.93; 0.90–0.97; p<0.01. The random effects model results in OR=0.70; 0.44–1.10; p=0.13, indicating that the results are unstable.

Table 1. Characteristic of included studies

Study	Study country (study design)	Sample size (study setting)	Gender (M/F)	Age (Year)	Screening parameter or tools	Prevalence of malnutrition	The stage of CKD	Influence factorsa	Quality
Guligowska *et al.* (2020)	Austria, Germany, Israel, Italy, The Netherlands, Poland, Spain (Cohort)	2,151 (Outpatient)	932/1,219	79.5±5.90	MNA and serum albumin	44.00%	Stage 2–5	4, 13	8
Lynch *et al.* (2013)	America (Cohort)	1,846 (Center)	764/981	57.8±14.10	Dry weight, mid-arm muscle circumference, and triceps skinfold thickness.	-	Stage 5	14	8
Windahl *et al.* (2018)	Germany, Italy, Netherland, Poland, Sweden, United Kingdom (Cohort)	1,334 (Clinics)	874/460	76	7-point SGA	21.00%	Stage 4–5	1, 3, 9, 15	6
Boaz *et al.* (2019)	Israel (Cross-secrtion-al)	378 (Center)	197/181	-	BMI and serum albumin	46.30%	Stage 5	6, 10,16	5
Dahl *et al.* (2021)	Norway (Cross-scrtional)	53 (Hospital)	39/14	62	NRS2002	26.00%	Stage 5	9, 17, 18	6
Czira *et al.* (2011)	Hungary (Cross-sectional)	973 (Outpatient)	555–418	51±13.00	MIS	28.50%	Stage 5	3	5
Hwang *et al.* (2009)	Korea (Cross-sectional)	110 (Outpatient)	46/64	58.6±1.00	Triceps skinfold thickness and Mid-arm muscle circumference	42.70%	Stage 5	19	4
Namuyimbwa *et al.* (2018)	Uganda (Cross-sectional)	182 (Outpatient clinic and wards)	56/126	33	BMI, serum albumin, Mid upper arm circumference	68.60%	Stage 1–5	1, 6, 20, 21, 22, 23	8
Omari *et al.* (2019)	Palestine (Cross-sectional)	174 (Hospital)	91/83	57.7±12.80	MIS	65.00%	Stage 5	1, 2, 7, 11, 24	7
Zhu *et al.* (2020)	China (Cross-sectional)	278 (Hospital)	163/115	68.78±4.23	PG-SGA	40.65%	Stage 5	3, 7, 10, 11, 25	5
Wang *et al.* (2012)	China (Cross-sectional)	125 (Hospital)	79/46	68.2±4.70	SGA	48.80%	Stage 5	2, 8, 9	6
Wang *et al.* (2016)	China (Cross-sectional)	114 (Hospital)	71/43	51.63±13.26	MQSGA	48.20%	Stage 5	1, 4, 5, 7 , 8	5
Li *et al.* (2018)	China (Cross-sectional)	72 (Hospital)	39/33	42.14±11.50	SGA	79.17%	Stage 5	4, 8	5

-: Not provided

[a]1: Age; 2: Comorbidites; 3: Depression; 4: Low Blood Albumin (ALB); 5: Creactive Protein (CRP); 6: Hemoglobin (Hb); 7: Long dialysis duration; 8: Inadequate dialysis; 9: Protein intake; 10: Requires feeding assistance; 11: Living status (with family); 12: Creatinine; 13: estimated Glomerular Filtration Rate (eGFR); 14: Taste perception; 15: Sex (Female); 16: Increased delivered dialysis dose; 17: Energy intake; 18: Pre-albumin; 19: Family history of chronic renal failure; 20: Being single; 21: Catholic religion; 22: CKD stage; 23: Low Density Lipoproteins (LDL); 24: The number of chronic medications; 25: Dialysis frequency

BMI: Body Mass Index; NRS2002: Nutritional Risk Screening 2002; MIS: Malnutrition–Inflammation Score; SGA: Subjective global assessment; PG-SGA: Scored patient-generated subjective global assessment; MQSGA: Modified Quantitative Subjective Global Assessment

Table 2. Meta-analysis of influence factors for malnutrition in CKD patients

Factors	n	Heterogeneity test		Model	OR	95% CI	*p*
		I^2 (%)	*p*				
Age	5 (Namuyimbwa *et al.* 2018; Omari *et al.* 2019; Wang *et al.* 2016; Windahl *et al.* 2018; Hwang *et al.* 2009)	87	<0.01	Random	1.29	(1.03–1.61)	0.02
Requires feeding assistance	2 (Boaz *et al.* 2019; Zhu *et al.* 2020)	0	0.70	Fixed	3.33	(2.55–4.35)	<0.01
Living status (with family)	2 (Omari *et al.* 2019; Zhu *et al.* 2020)	0	0.49	Fixed	0.49	(0.34–0.71)	<0.01
Protein intake	3 (Wang *et al.* 2012; Windahl *et al.* 2018; Dahl *et al.* 2022)	0	0.97	Fixed	0.89	(0.85–0.94)	<0.01
Comorbidities	2 (Omari *et al.* 2019; Wang *et al.* 2012)	0	0.44	Fixed	1.78	(1.03–3.07)	0.04
Long dialysis duration	3 (Omari *et al.* 2019; Wang *et al.* 2016; Zhu *et al.* 2020)	0	0.64	Fixed	1.61	(1.16–2.24)	<0.01
Inadequate dialysis	3 (Li *et al.* 2018; Wang *et al.* 2016; Wang *et al.* 2012)	60	0.08	Random	1.25	(1.12–1.40)	<0.01
ALB	3 (Guligowska *et al.* 2020; Li *et al.* 2018; Wang *et al.* 2016)	94	<0.01	Random	0.70	(0.44–1.10)	0.13
Hb	2 (Boaz *et al.* 2019; Namuyimbwa *et al.* 2018)	62	0.11	Random	1.84	(0.92–3.66)	0.08
Depression	3 (Boaz *et al.* 2019; Czira *et al.* 2011; Namuyimbwa *et al.* 2018)	0	0.55	Fixed	3.44	(2.21–5.34)	0.04

ALB: Low Blood Albumin; Hb: Hemoglobin

Protective factors against malnutrition

Protein intake was demonstrated to a protective risk factor of malnutrition among CKD patients according to our analysis (Wang *et al.* 2012; Windahl *et al.* 2018; Dahl *et al.* 2022). However, given the prolonged duration of CKD, patients with enduring dietary limitations often harbor concerns about protein consumption. They tend to instinctively avoid high-protein foods, even when their dietary restrictions post-dialysis are not as stringent (Kiuchi *et al.* 2016). Low protein diets could retard the progression of CKD but worsened the nutritional status of patients (Noce *et al.* 2016).

The review incorporates two studies that have demonstrated living with family as a protective factor against malnutrition among CKD patient (Omari *et al.* 2019; Zhu *et al.* 2020). Silva *et al.* (2016) emphasized the significant impact of family and social support on the physical and mental health of individuals with CKD, consequently affecting their nutritional status (Silva *et al.* 2016). Additionally, the current research highlights that emotional and financial support provided by family members can enhance quality of life, foster healthy behaviors, improve adherence to nutritional guidance, and decrease the risk of malnutrition among patients (Kiajamali *et al.* 2017).

Risk factors for malnutrition

Among the various studies incorporated in the review, five investigations have consistently demonstrated that age stands out as a prominent risk factor for malnutrition among CKD patients (Namuyimbwa *et al.* 2018; Omari *et al.* 2019; Wang *et al.* 2016; Windahl *et al.* 2018; Hwang *et al.* 2009). Notably, older CKD patients face a higher susceptibility to malnutrition compared to other age groups. These reasons include dietary

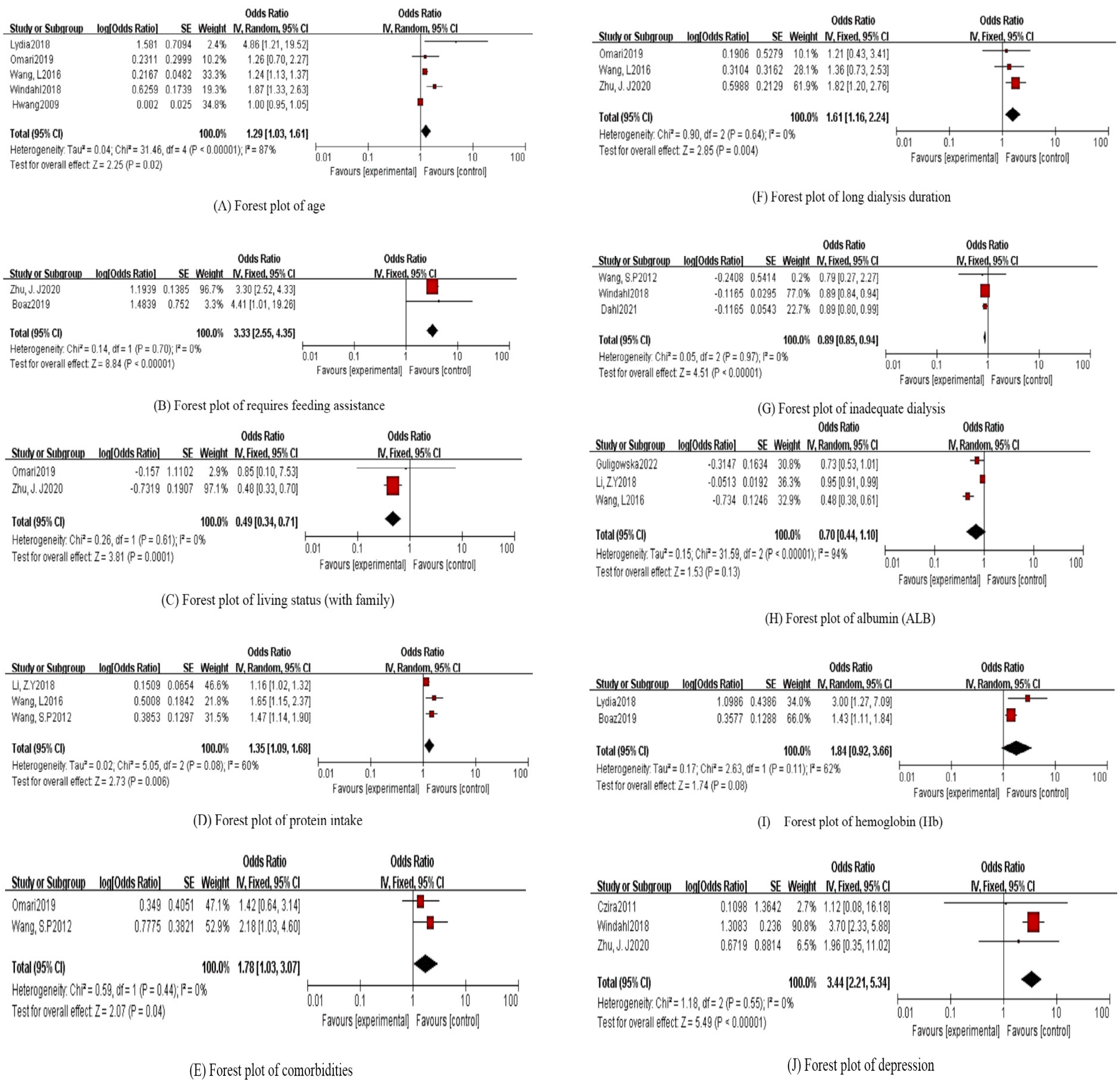

Figure 2. Forest plot of meta-analysis of influcing factors for malnutrition in CKD patients

restrictions, frailty, cognitive decline, diminished gastrointestinal function, tooth loss, reduced appetite, deteriorated taste perception (Kiuchi *et al.* 2016).

CKD patients' condition of requiring feeding assistance was reported as a risk factor for malnutrition based on two studies (Zhu *et al.* 2020; Boaz *et al.* 2019). Generally, this condition indicates poor independent living skills. Furthermore, patients exhibiting better independent living skills tended to take a more proactive approach in maintaining and enhancing their own health (Zhu *et al.* 2020).

Comorbidities have been identified as risk factors for malnutrition in CKD patients (Omari *et al.* 2019; Wang *et al.* 2012). Rhee *et al.* (2015) demonstrated that among patients with nephropathy, inadequate glycemic control independently correlated with markers indicative of malnutrition (Rhee *et al.* 2015). Similar associations were found that diabetics undergoing hemodialysis exhibited reduced food intake compared to non-diabetic counterparts, directly contributing to compromised nutritional status (Bataille 2017).

Table 3. Sensitive analysis of influence factors for malnutrition in CKD patients

Factors	Fixed model			Random model		
	OR	95% CI	*p*	OR	95% CI	*p*
Age (Namuyimbwa *et al.* 2018; Omari *et al.* 2019; Wang *et al.* 2016; Windahl *et al.* 2018; Hwang *et al.* 2009)	1.06	(1.02–1.11)	<0.01	1.29	(1.03–1.61)	0.02
Protein intake (Wang *et al.* 2012; Windahl *et al.* 2018; Dahl *et al.* 2022)	0.89	(0.85–0.94)	<0.01	0.89	(0.85–0.94)	<0.01
Requires feeding assistance (Boaz *et al.* 2019; Zhu *et al.* 2020)	3.33	(2.55–4.35)	<0.01	3.33	(2.55–4.35)	<0.01
Living status (with family) (Omari *et al.* 2019; Zhu *et al.* 2020)	0.49	(0.34–0.71)	<0.01	0.49	(0.34–0.71)	<0.01
Comorbidities (Omari *et al.* 2019; Wang *et al.* 2012)	1.78	(1.03–3.07)	0.04	1.78	(1.03–3.07)	0.04
Long dialysis duration (Omari *et al.* 2019; Wang *et al.* 2016; Zhu *et al.* 2020)	1.61	(1.16–2.24)	<0.01	1.61	(1.16–2.24)	<0.01
Inadequate dialysis (Li *et al.* 2018; Wang *et al.* 2016; Wang *et al.* 2012)	1.25	(1.12–1.40)	<0.01	1.35	(1.09–1.68)	<0.01
ALB (Guligowska *et al.* 2020; Li *et al.* 2018; Wang *et al.* 2016)	0.93	(0.90–0.97)	<0.01	0.70	(0.44–1.10)	0.13
Hb (Boaz *et al.* 2019; Namuyimbwa *et al.* 2018)	1.52	(1.19–1.93)	<0.01	1.84	(0.92–3.66)	0.08
Depression (Boaz *et al.* 2019; Czira *et al.* 2011; Namuyimbwa *et al.* 2018)	3.44	(2.21–5.34)	0.04	3.44	(2.21–5.34)	<0.01

Lengthy dialysis duration has identified as a notable risk factor for malnutrition in CKD patients across three studies (Omari *et al.* 2019; Wang *et al.* 2016; Zhu *et al.* 2020). The catabolic process, which accelerates the significant of large amounts of nutrients, may explain the higher prevalence of malnutrition during extended dialysis duration (Jankowska *et al.* 2017). Salame *et al.* (2018) emphasized that extended periods of dialysis lead to significant protein and amino acid loss, and if not replaced in a timely manner, the patient's nutritional status deteriorates over time (Salame *et al.* 2018).

Inadequate dialysis stands as one of the risk factors for malnutrition among CKD patients in three studies (Li *et al.* 2018; Wang *et al.* 2016; Wang *et al.* 2012). The high incidence of malnutrition in patients undergoing inadequate dialysis may be attributed to the ineffective removal of uremic toxins, resulting in altered taste sensations among patients, affecting appetite, food intake, consequently resulting in malnutrition (Hara *et al.* 2018). Therefore, patients receiving dialysis should be provided with the recommended dialysis dose to avoid inadequate treatment (Iorember 2018).

Two different studies (Boaz *et al.* 2019; Namuyimbwa *et al.* 2018) within this review have consistently identified Hb as a risk factor for malnutrition. Their findings revealed a substantial association between hemoglobin levels below 11.5 g/dL and a threefold higher prevalence of malnutrition when contrast to individuals with hemoglobin levels at or above 11.5 g/dL (Namuyimbwa *et al.* 2018). The discrepancy might stem from malnourished CKD patients experiencing reduced bone marrow responsiveness to erythropoietin (Weir 2021).

Depression has emerged as a significant risk factor for malnutrition in CKD patients based on findings from three studies (Boaz *et al.* 2019; Czira *et al.* 2011; Namuyimbwa *et al.* 2018). Gebrie & Ford (2019) highlighted that patients with CKD frequently experience negative mood states, and as renal function deteriorates, the incidence of depression tends to rise (Gebrie & Ford 2019). A pivotal link between depression and malnutrition exists due to the common manifestation of reduced appetite, a prevalent symptom of depression. Consequently, as patients consume less food, the likelihood of malnutrition escalates (Türk *et al.* 2020).

Strengths and limitations of the review

The key strength of this study lies in its pioneering role as the first systematic review and meta-analysis investigating the factors influencing malnutrition in individuals with CKD. Additionally, all articles included in this review/ meta-analysis can be considered as moderate to high quality studies, enhancing the credibility and reliability of the findings.

Nonetheless, several limitations must be acknowledged. Firstly, certain influencing factors were only documented in a limited number of studies, precluding their incorporation into a meta-analysis to establish their impact on malnutrition. Additionally, high heterogeneity was observed in the results of some studies, as a result of differences in the study populations.

Implications for clinical practice

The early detection and recognition of malnutrition among CKD patients by clinicians and nursing staff hold significant importance within clinical practice. Effective identification of malnourished patients and subsequent enhancement of their nutritional status hinge on addressing the associated factors of malnutrition and promptly initiating targeted interventions. Concurrently, there is a call for diligent exploration into the mechanisms through which various factors contribute to the emergence of malnutrition in CKD patients.

CONCLUSION

This meta-analysis delved into the array of factors influencing malnutrition among CKD patients. The findings underscore that several factors, including age, the need for feeding assistance, living arrangements (with family), protein intake, comorbidities, prolonged dialysis duration, inadequate dialysis, Hemoglobin (Hb) levels, and depression, significantly contribute to the risk of malnutrition among CKD patients. Notably, living with family and adequate protein intake emerge as protective factors against malnutrition within this patient population. Moreover, this review did not confirm ALB as a risk factor for malnutrition. Thus, it's crucial to conduct prospective studies with larger sample sizes to conclusively validate or refute this result.

ACKNOWLEDGEMENT

We acknowledge the efforts of each and every member of the research team

DECLARATION OF CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2024.19.1.61-68*
ISSN 1978-1059 EISSN 2407-0920

Effect of Sacha Inchi Oil on Human Blood Pressure and Lipid Profile: A Preliminary Study in Malaysia

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ABSTRACT

This uncontrolled pre-post study aimed to assess changes in blood lipid profiles and blood pressure resulting from a 2-month consumption of Sacha Inchi Oil (SIO) *(Plukenetia volubilis)*. Investigating the effects of SIO on Malaysians is essential for tailoring interventions to the local context, considering genetic, cultural, and dietary differences. A total of 13 adult participants, comprising 8 males and 5 females aged 35 to 74 years old, took part in this intervention study. Each participant was instructed to consume 2 softgels daily, with each softgel containing 530 mg of SIO. Blood lipid profiles and blood pressure were measured at baseline and at the end of the 2-month period, utilizing venipuncture for blood tests and a sphygmomanometer for blood pressure assessment. The results indicated a significant reduction in Systolic Blood Pressure (SBP) levels following SIO supplementation (2 softgels daily) ($p<0.05$). However, there were no notable improvements in Diastolic Blood Pressure (DBP), Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C), and Triglycerides (TG) after the 2-month supplementation. In conclusion, the daily supplementation of 2 softgels of SIO (1,060 mg) for 2 months demonstrated a beneficial effect on blood pressure, particularly in reducing SBP. These findings serve as preliminary data for future research into the potential health benefits of SIO in the Malaysian population.

Keywords: blood lipid profile, blood pressure, sacha inchi oil

INTRODUCTION

Sacha inchi (*Plukenetia volubilis*) is an indigenous plant of the Euphorbiaceae family, originates from the Peruvian rainforest and is cultivated at altitudes ranging from 200 to 2,000 meters above sea level, thriving within a temperature range of 10 to 37°C (Cárdenas *et al.* 2021; Kodahl & Sørensen 2021). Traditionally used by indigenous Peruvians for medicinal purposes, its leaves were employed to address rheumatoid issues (Chirinos *et al.* 2013) and as a remedy for burns (Lock *et al.* 2016). Recently, its nutritional value has garnered attention, particularly for its rich protein and lipid content, with seeds comprising approximately 25–30% protein and 35–60% lipids (Chirinos *et al.* 2013). Commercially cultivated primarily for its oil, Sacha inchi serves as a notable source of Polyunsaturated Fatty Acid (PUFA), Alpha Linolenic Acid (ALA), and Linoleic Acid (LA), as well as monounsaturated fatty acids, particularly Oleic Acid (OA) (Cárdenas *et al.* 2021). The omega-6 to omega-3 ratio in Sacha Inchi Oil (SIO) approximates 1:1.3 (Carrillo *et al.* 2018), aligning closely with the recommended target ratio of 1:1 to 2:1 (Simopoulos 2002).

Recent evidence highlights the potential health benefits of SIO, including its ability to lower blood pressure and improve lipid profiles (Garmendia *et al.* 2011; Huamán Saavedra *et al.* 2012; Gonzales & Gonzales 2014). Studies suggest that ALA found in SIO possesses anti-inflammatory properties by inhibiting pro-inflammatory cytokines such as Interleukin (IL) and Tumor Necrosis Factor-Alpha (TNF-α), and improving endothelial function (Erdinest *et al.* 2012; Zhao *et al.* 2004; Minami *et al.* 2017). Since endothelial dysfunction is a known marker for Cardiovascular Diseases (CVDs), enhancing endothelial function may help reduce the risk of CVDs (Barthelmes *et al.* 2017).

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(Received 18-12-2023; Revised 28-02-2023; Accepted 19-03-2024; Published XX-03-2024)

Most studies on SIO focus on its benefits for Non-communicable Diseases (NCDs), especially Cardiovascular Diseases (CVDs), predominantly in South America, particularly Peru. However, there's a lack of research on SIO's effects in Asia, notably in Malaysia. Understanding SIO's impact on Malaysians is crucial for tailoring interventions considering genetic, cultural, and dietary differences. This study aims to explore how these factors influence SIO's outcomes, recognizing genetic variations' role in nutrient metabolism (Al-Jardli & Elizabeth 2023). Examining SIO within the Malaysian diet can offer insights into integrating it into local cuisines and diets, hence promotes culturally acceptable and sustainable interventions.

A recent health and morbidity survey conducted in Malaysia revealed a high prevalence of NCDs such as hypercholesterolemia, hypertension, diabetes, and metabolic syndrome (WHO 2021). These NCDs are closely associated with abnormal lipid profiles and blood pressure, which can be exacerbated by physical inactivity and an unhealthy diet (WHO 2010). Therefore, nutrition interventions play a critical role in addressing issues related to abnormal blood lipid and blood pressure levels to reduce the risks of NCDs. Currently, there is a lack of research on nutritional interventions for these health challenges in Malaysia. To the best of our knowledge, this is the first nutritional epidemiological study investigating the health effects of SIO in Malaysia, aiming to bridge the research gap within the Asian population.

METHODS

Design, location, and time

A non-probability purposive sampling method was applied to select study participants. Recruitment of participants was carried out through 3-month advertisements within a local community by a nutraceutical manufacturer company, none were referred directly by a physician. The company advertised this study of two months SIO supplementation by distributing leaflets through social media including the company's website and Facebook. The target audience would typically include individuals who are interested in health and wellness, dietary supplements, and improving their overall well-being. Online advertising was considered to reduce face-to-face contact and risks of COVID-19 infection during the COVID-19 pandemic. The leaflet consisted of information about the study objectives, inclusion and exclusion criteria, study period, benefits and risks of SIO supplementation, and contact number of the researcher.

This uncontrolled pre-post study utilized a quasi-experimental design, involving 8 male and 5 female adults aged 35 to 74 years old (n=13). To determine the minimum required sample size, findings from a previous study on the effects of SIO consumption were consulted (Garmendia *et al.* 2011). To detect an effect size of Cohen's d=1.73 with 95% power (alpha=0.01, two-tailed), a paired sample t-test calculation using G*Power software (Faul *et al.* 2007) indicated that a minimum of 10 participants would be necessary. Therefore, considering an estimated dropout rate of 20%, a minimum of 12 participants was deemed appropriate. This intervention was conducted from January to March 2022 in Ipoh, Perak. Research approval was granted by the Human Research Ethics Committee of Universiti Sains Malaysia (JEPeM Code: USM/JEPeM/21060463).

Materials and tools

Participants completed a self-administered questionnaire designed to gather sociodemographic information. This questionnaire incorporated a mix of closed-ended and open-ended questions to capture data pertaining to various sociodemographic factors, including age, gender, ethnicity, educational attainment, and details regarding smoking and alcohol consumption habits. Participants also provided self-reported measurements for both weight and height, which were subsequently utilized to compute their Body Mass Index (BMI) using the formula: (weight in kg)/(height in m)^2. Following this calculation, participants were categorized into one of the following BMI groups, consistent with the World Health Organization guidelines (WHO 2010): underweight (<18.5 kg/m^2), normal weight (18.5 to 24.9 kg/m^2), overweight (25.0 to 29.9 kg/m^2), or obese (≥30.0 kg/m^2).

The Diet History Questionnaire (DHQ) was originally developed by Shahar *et al.* (2000) and Suzana *et al.* (2011). It served as the tool for evaluating food and dietary supplement consumption at both the study's commencement and its conclusion, spanning the 2-month duration. Participants were interviewed via telephone conversations and provided with guidance to recall their weekly food consumption. The data on nutrient intake was subsequently analyzed using Nutritionist Pro software Version 7.9.

International Physical Activity Questionnaire-Short Form (IPAQ-SF) was developed by Craig *et al.* (2003). It is a self-administered questionnaire consisting of 7 items aimed at capturing information regarding the frequency and duration of physical activities across different intensity levels. This questionnaire was administered both at the initiation and conclusion of the 2-month study period. Participants' physical activity levels were subsequently categorized as high, moderate, or low, following the scoring protocol established for the IPAQ-SF.

Procedures

Eligible participants were non-vegetarian adults aged 30 or older, with a BMI ranging from >20 to <35 kg/m^2. They had to exhibit at least one medical condition diagnosed by Malaysian medical service as borderline high blood lipid profiles or prehypertension. Participants with chronic diseases such as cancer, kidney or liver diseases, psychiatric disorders, severe heart failures, autoimmune diseases, cerebrovascular diseases, uncontrolled hypertension, or pregnant women were excluded from the study.

A total of 13 eligible participants willingly enrolled in this intervention study and provided informed consent. They had successfully adhered to the SIO supplementation for 2 months, resulting in a zero dropout rate. Besides completing the questionnaires, they were assigned to receive daily supplementation with SIO softgels as part of the research procedure, with each softgel containing 530 mg of SIO. Participants were instructed to take one softgel in the morning after breakfast and another softgel at night after dinner, adhering to this regimen for a duration of 2 months. Notably, each softgel consisted of 228 mg of ALA (Omega 3), 170 mg of LA (Omega 6), and 48 mg of OA (Omega 9). The formulation of the softgels was established based on the average specifications of the raw ingredients (Certificate of Analysis #2019004, Agroindustrial Osho SAC) and had received approval from the National Pharmaceutical Regulatory Agency in Malaysia.

To ensure compliance, participants' adherence to the supplementation schedule was closely monitored through regular follow-up calls. Throughout the study, participants were encouraged to maintain their customary dietary habits and level of physical activity to mitigate potential confounding effects on the study's outcomes. Meanwhile, participants were divided into strata based on their medication use to control the confounding effects of medications.

Blood pressure and biochemistry assessments including Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C), and Triglycerides (TG), were obtained through blood tests conducted at both the baseline and the end of the 2-month intervention period. The 13 participants were instructed to visit an established and registered clinical laboratory in Ipoh to undergo these tests and have their blood pressure assessed. A trained technician at the laboratory performed blood draws using venipuncture and measured blood pressure using a sphygmomanometer. Biochemical analysis was carried out using the Atellica® CH Analyzer. The results of these clinical assessments were then sent to the researcher via email.

Data analysis

The data obtained were analyzed using IBM Statistical Package for the Social Sciences software (SPSS) Version 26.0. Descriptive statistics were employed to analyze the sociodemographic data, which were presented in terms of frequency (n) and percentage (%). Prior to analysis, DHQ data was processed using Nutritionist Pro software to estimate total energy and macronutrient intake for the participants. To compare the mean differences in physical activity, dietary intake, blood profile, and blood pressure before and after the intervention, paired t-tests were performed. The confidence interval and significance level for the paired t-tests were set at 95% and 0.05, respectively.

RESULTS AND DISCUSSION

Demographic and body weight status

The demographic characteristics of the participants are summarized in Table 1. The mean age of the participants was 55.23±9.23 years, with a higher proportion of males (61.5%) compared

Table 1. Participants' sociodemographic characteristics (n=13)

Characteristic	Mean (SD)/Median (IQR)
Age (years)	55.23 (9.23)
Height (m)[a]	1.61 (0.12)
Weight (kg)	
Baseline	75.89 (22.44)
After 2 month	75.42 (22.26)
BMI (kg/m^2)[a]	
Baseline	26.85 (7.58)
After 2 months	26.56 (7.89)

[a] : median and IQR were reported; BMI: Body Mass Index

to females (38.5%). There was no statistically significant difference in BMI observed before (26.85±7.58) and after (26.56±7.89) the intervention (p>0.05).

Medication history and lifestyle behaviors

Six participants (46.2%) were taking medications prescribed by doctors. These medications were primarily for high blood pressure (n=6), high blood cholesterol and triglycerides (n=4), gout (n=1), diabetes (n=3), prevention of rejection after kidney transplantation (n=1), and treatment of skin disorders (n=1). Some participants were on multiple types of medications.

Among the 13 participants, there were 2 smokers (15.4%), but none of them consumed alcohol. Throughout the 2-month intervention period, all participants maintained their smoking behavior without any changes. The comparison of energy and macronutrient intake by the participants before and after the intervention revealed no significant differences (p>0.05) (Table 2). It suggests that participants adhered to the dietary instructions provided. This strengthens the validity of the study results by reducing the likelihood that changes in dietary habits confounded the observed effects of sacha inchi oil on blood pressure and lipid profile. However, it is important to recognize potential limitations, such as self-reporting biases in dietary assessments or unaccounted-for variations in food intake that may still exist despite efforts to control them. Nonetheless, addressing these potential confounders enhances the strength of the study's conclusions.

Additionally, all participants fell into either moderate or low level of physical activity categories, with median values of 531 MET-min/week at baseline and 693 MET-min/week after 2 months. Wilcoxon signed-rank test showed no significant changes in the total energy expended for various activities (total MET-min/week) before and after the 2-month intervention period (Z statistic=0.051, p=0.959).

Effect of SIO supplementation on blood lipid profile

The changes in blood lipid profiles (TC, LDL-C, HDL-C, and TG) before and after a 2-month SIO intervention are depicted in Figure 1. Despite the absence of significant changes, the levels of TC and LDL-C showed attenuation by the end of the intervention period, while HDL-C had a slight increase. However, the median TG level remained at 2.00 mmol/L after 2 months of SIO supplementation.

Our findings indicate that a 2-month intervention of SIO supplementation did not have a significant impact on TG levels, aligning with the result of earlier researches (Garmendia *et al.* 2011; Gonzales & Gonzales 2014). However, it contradicts the findings of a quasi-experimental study that reported a significant reduction in

Table 2. Changes of mean energy and macronutrients intake during intervention period (n=13)

Variable	Mean difference (95% CI)	t statistics (df)	*p*[a]
Energy (kcal)	16.01 (-140.13, 172.14)	0.223 (12)	0.827
CHO (g)	-0.44 (-24.88, 24.01)	-0.039 (12)	0.970
Protein (g)	2.87 (-1.57, 7.30)	1.409 (12)	0.184
Fat (g)	4.65 (-5.55, 6.48)	0.168 (12)	0.869

[a]: Tested using paired t-test

postprandial triglyceridemic levels among 12 young adults who consumed 50 g of sacha inchi (Huamán *et al.* 2008). Similarly, Huamán Saavedra *et al.* (2012) found that a 30 g ingestion of sacha inchi over 6 weeks led to a significant reduction in triglycerides, total cholesterol, and LDL cholesterol levels among 14 young adults. Our findings do not align with several previous studies investigating the effects of SIO on lipid profiles in both animal and human subjects. Those studies typically involved longer intervention periods (3 to 4 months) and supplementation doses of 5, 10, or 20 mL of SIO (Garmendia *et al.* 2011; Gonzales & Gonzales 2014). Therefore, the lack of a significant impact on blood lipid profiles in our study may be attributed to the lower dose of SIO supplementation or the relatively short intervention period.

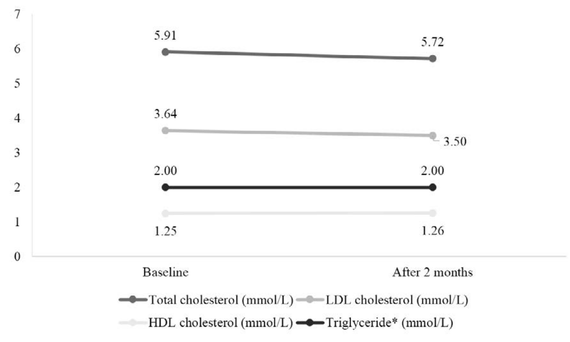
The hypolipidemic effects of SIO may be attributed to its high content of ALA (alpha-linolenic acid). Several studies have demonstrated the impact of ω-3 fatty acids on improving blood lipid profiles (Preston Mason 2019). While the detailed mechanisms are not fully understood, ω-3 fatty acids are believed to reduce LDL, LDL-apoprotein-B, Triglyceride (TG) production, and the incorporation of TG into VLDL (very low-density lipoprotein) (Tall & Yvan-Charvet 2015; Backes *et al.* 2016). Furthermore, by reducing the expression of the transcriptional factor SREBPs (sterol regulatory element-binding proteins), ALA is expected to inhibit the cholesterol and fatty acid biosynthesis pathway (Fukumitsu *et al.* 2013).

Effect of SIO supplementation on blood pressure

Figure 2 shows SBP declined significantly after the 2-month period ($p<0.05$) but DBP did not show significant change.

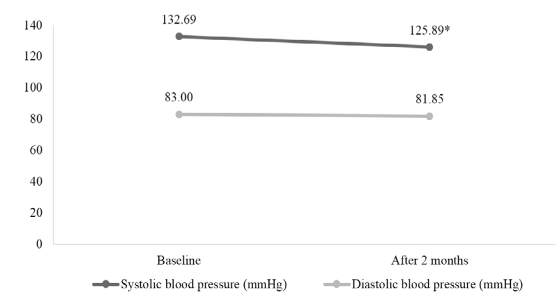
This study revealed that SIO supplementation for 2 months significantly reduced SBP, decreasing it from 132.69 mmHg to 125.89 mmHg ($p<0.05$). These findings are in line with a study by Gonzales and Gonzales (2014), which reported that treatment with 10 or 15 mL of SIO significantly reduced both SBP and DBP during the second month of the intervention period. A similar pattern was also observed in another study involving L-NAME-induced hypertensive Holtzmann rats (Gorriti Gutierrez & Quispe 2010), which revealed that SIO at doses of 0.1, 0.5, and 1 mL exerted a hypotensive effect. Doses of 0.5 ml and 1 mL were more effective in reducing SBP, while doses of 0.1 mL and 1 mL showed a similar lowering effect on DBP. The blood pressure-lowering effect of SIO may be attributed to its high content of polyunsaturated fatty acids, primarily ALA and LA, which account for approximately 47.04% and 34.98% of the total fat, respectively (Carrillo *et al.* 2018).

Dietary polyunsaturated fatty acids have been shown to have a positive impact on blood pressure (Grynberg 2005). However, Djoussé *et al.* (2005) suggested in their study that dietary linolenic acid (ALA), but not LA, was associated with a lower incidence of high blood pressure. They concluded that ALA was responsible for significantly reducing SBP but not DBP.



*Median values were presented

Figure 1. Changes of mean/median blood lipid profile before and after the intervention (n=13)



*SBP decreased significantly after 2-month period [$t = 2.628$ (12), $p<0.05$]

Figure 2. Changes of mean blood pressure before and after the intervention (n=13)

Meanwhile, Minami *et al.* (2017) demonstrated in their study that SIO supplementation improved endothelial function by increasing Flow-Mediated Vasodilation (FMD). Several studies have indicated that endothelial function is impaired in animal models of high blood pressure (Tsutsui *et al.* 2010). Moreover it also been suggested that vascular endothelial dysfunction may contribute to the development of high blood pressure (Rajendran *et al.* 2013).

To assess the potential confounding effect of medication, a stratification analysis was conducted. Figure 3 provides evidence that the effect of SIO on SBP was not influenced by the participants' medication status. In fact, a paired sample t-test demonstrated a significant reduction in SBP after the 2-month intervention among the 7 participants who were not taking any medication (t=3.440, p=0.014). This reduction suggests that SIO independently contributed to lowering SBP in this subgroup. SIO might possess bioactive components that directly influence blood pressure regulation. These components could affect physiological pathways involved in SBP regulation, leading to a reduction in blood pressure levels (Djoussé *et al.* 2005).

While the effect of SIO on SBP was not influenced by medication status overall, it's possible that interactions between SIO and certain medications may have masked or altered its effects in some participants. Overall, these findings suggest that SIO has a beneficial effect on SBP, especially among individuals who are not taking any medication. However, further research is needed to understand the mechanisms underlying this effect and to confirm its generalizability to broader populations.

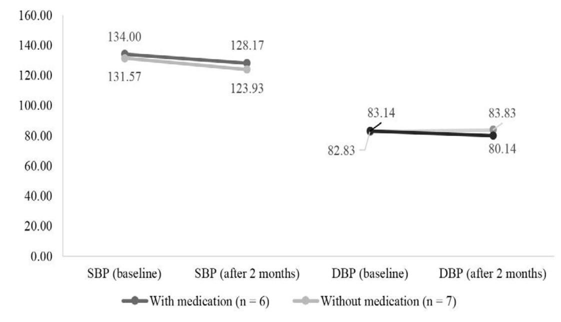


Figure 3. Comparison of mean blood pressure changes in 2 months between participants with and without medication

Limitation of study

Despite the small sample size and quasi experimental design utilized, there are reasons supporting these choices. Resource constraints and ethical challenges during the pandemic necessitated this initial investigation to explore the feasibility of conducting further research on SIO in the future. This study serves to refine study protocols based on initial findings and aims to generate preliminary evidence rather than definitive conclusions. However, lacking a control group and limited control over confounding variables result in relatively low levels of evidence. Therefore, caution is advised when interpreting the findings, and their generalizability to other populations or settings may be limited due to differences in patient characteristics.

This study was conducted during the Malaysian Movement Control Order (MCO) in response to the COVID-19 pandemic. Participants self-reported their body height and weight, potentially introducing information bias, although BMI was not the primary outcome. Despite this, further analysis found no significant changes in energy and macronutrient intake, as well as energy expenditure levels during the 2-month intervention period. This helped address the confounding effects. Additionally, stratification analysis showed that participants' medication status did not confound the effect of SIO on SBP.

Despite these limitations, this study offers valuable insights into intervention outcomes, particularly in real-world settings where participant randomization or use of control group may be ethically challenging

CONCLUSION

The current study indicates that a 2-month daily supplementation of SIO (2 x 530 mg softgel) significantly reduces the Systolic Blood Pressure (SBP) of the participants. While acknowledging the limitations of a small sample size and quasi-experimental design, these findings serve as valuable starting point, providing initial insights that can inform the development of more robust research in the future.

Therefore, it is advisable to plan future studies using a Randomized Controlled Trial (RCT) design with a larger sample size and higher

SIO doses. Additionally, a longer intervention period is clearly necessary to further validate these findings.

ACKNOWLEDGEMENT

The authors wish to express their profound appreciation to all the volunteers who participated in this study and generously dedicated their valuable time to this intervention research. They also extend their heartfelt gratitude to LinoGreen Sdn. Bhd (https://linogreen.com/en/) for providing the funding that made this research possible.

DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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