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Dyslipidemia in Renal Dysfunction among Non-diabetic Individuals from the 2019 Indonesian Cohort Study: A Cross-Sectional Study

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ABSTRACT

The aim of this study was to investigate the relationship between dyslipidemia and the estimated Glomerular Filtration Rate (eGFR) values in a healthy population without a history of diabetes mellitus. Data were part of the cohort study database of 2019. Data analysis was perfomed using descriptive and inferential statistics with linear regression in 893 of 1,545 non-diabetic participants. The results showed that the average cholesterol levels, High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), and triglycerides were 196.75, 48.71, 123.37, and 109.56 mg/dl, respectively, and the average eGFR level of the respondents was 98.47±15.50 mg/dl. This study found that age, HDL levels, and LDL levels had a significant relationship with eGFR ($p<0.05$). It was concluded that increasing age and LDL levels and decreasing HDL levels would decrease eGFR.

Keywords: dyslipidemia, glomerular filtration rate, non-diabetes

INTRODUCTION

The incidence of non-communicable diseases is increasing in developing countries; one of these diseases is Chronic Kidney Disease (CKD), often refrred to as chronic kidney failure. Based on data from the Basic Health Survey in Indonesia, which was identified using respondents' admissions and doctors' diagnoses of CKD, the incidence of this disease incidence has almost doubled, from 0.2% in 2013 to 0.38% in 2018 (Ministry of Health Republic of Indonesia (MoH RI 2013; 2018)).

Chronic kidney disease is a condition in which the kidneys have difficulty filtering blood due to kidney damage. Based on the level of kidney function, there are five stages that describe the condition of CKD. The staging of CKD is determined by estimating the Glomerular Filtrate Rate (eGFR), which is measured by how well the kidneys filter the blood. Kidney disease is divided into five main stages, ranging from stage 1 (mild) to stage 5 (kidney failure). The staging category is determined based on eGFR as follows: eGFR values greater than or equal to 90 ml/minute /1.73 m^2 indicates stage 1 (mild kidney damage), eGFR of 60–89 ml/minute /1.73 m^2 is an indication of stage 2 (slight loss of kidney function), while stages 3a & 3b (eGFR of 30–59 ml/min /1.73 m^2) are classified as mild to severe loss of kidney function, stage 4 is when the kidney is in a state of severe decompensation (eGFR of 15–29 ml/min/1.73 m^2), and stage 5 is a condition of renal failure or near renal failure (eGFR of less than 15 ml/min/1.73 m^2) (Chen *et al.* 2013; Theofilis *et al.* 2021).

Several methods of calculating eGFR have been studied. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) calculation method is generally more accurate than the Modification of Diet in Renal Disease (MDRD) eGFR calculation method (Levey & Stevens 2010). The CKD-EPI calculation method is also more accurate than the MDRD method for assessing eGFR levels in healthy populations in Asia without a history of diabetes, hypertension, and kidney disorders (Teo *et al.* 2014).

The eGFR in chronic kidney disease can be influenced by various factors such as age, sex, serum creatinine level, diet, and several clinical parameters, such as blood glucose levels and lipid profiles (Arifa *et al.* 2017). Other clinical

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parameters such as dyslipidemia have also been proven to affect the kidneys, especially in individuals with obesity or overweight and hyperglycemia, because it will burden the work of the renal organs (Kurniati & Tahono 2016; Navratilova *et al.* 2020). Significant increases in total cholesterol, triglyceride, and low-density lipoprotein levels are frequently associated with an increased likelihood of a decreased estimated Glomerular Filtration Rate (eGFR) and the development of chronic kidney disease (Liang *et al.* 2020). In the early stages of CKD, disturbances in lipoprotein metabolism are evident and usually progress toward reduced kidney function. Dyslipidemia has a major influence on the pathogenesis of CVD and impaired kidney function (Tsimihodimos *et al.* 2011).

Dyslipidemia in people with kidney failure is characterized by increased levels of total cholesterol, Triglycerides (TGs), and Low-Density Lipoprotein Cholesterol (LDL-C) and decreased levels of High-Density Lipoprotein Cholesterol (HDL-C) (Adejumo *et al.* 2016). In addition, a higher Body Mass Index (BMI) has been associated in large population-based studies with the development of a low estimated Glomerular Filtration Rate (GFR), the development of End-Stage Renal Disease (ESRD), which can occur in people at any age, and a more rapid loss of estimated GFR over time. In individuals with a history of CKD, an increase in BMI or obesity class II or higher is considered a risk factor for the rapid development of CKD itself (Kovesdy *et al.* 2017).

Studies based on several parameters, including clinical chemistry, have been widely conducted to predict chronic kidney disease as characterized by a decreased eGFR value, but these studies are still rarely conducted on healthy people, especially the population who are the respondents in cohort studies in Indonesia (Sulistiowati & Idaiani 2015). Therefore, this study was conducted to analyze the relationship between several clinical chemistry parameters in predicting the decline of eGFR in the study population of the Non-Communicable Disease Risk Factors Cohort in Indonesia.

METHODS

Design, location, and time

This study is a cross-sectional study using data collected during the Biomedical Cohort Study of Risk Factors for Non-communicable Diseases in 2019, which sampled from five districts in the city of Bogor, West Java Province. The data collection was conducted from March to December 2019 and was approved by the Ethics Committee of the Research and Development Agency No.LB.02.01/2/KE. 120/2019.

Sampling

Inclusion criteria for this study were members of the target population aged over 25 years who did not have diabetes mellitus or prediabetes and who had fasting and 2-hour postprandial glucose test results. Exclusion factors were members of the target population who had diabetes mellitus or prediabetes and did not have complete glucose test results as required by the inclusion criteria. Another exclusion criterion was the condition of the lysed blood serum samples. There were 894 out of 1,545 respondents who did not have diabetes mellitus or prediabetes.

Data collection

The respondents in this research were women and men aged 28 years and above. They were interviewed for their health data using a questionnaire, had their blood drawn for analysis, and underwent other medical examinations.

The respondents' blood samples were processed by centrifugation to obtain serum, which was then analyzed using a clinical chemistry tool branded Pentra® for blood analysis parameters such as total cholesterol, LDL, HDL, triglycerides and serum creatinine. The measurement of clinical parameters was performed according to the Standard Operational Procedure (SOP), which had undergone internal and external validation tests, and was performed with the assistance of a clinical pathologist.

In this study, fasting and 2-hour postprandial blood glucose levels in the normal category, which are 70–99 mg/dl and 70–139 mg/dl, respectively, are based on categorization by the Indonesian Association of Endocrinologists (PERKENI 2021).

Lipid profile categorization in this study follows the guidelines issued by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III). Normal cholesterol level is <200 mg/dl, normal LDL is <100 mg/dl, normal triglyceride is <150 mg/dl, and high level of HDL is ≥40 mg/dl for men and ≥50 mg/dl for women. Estimated glomerular filtration rate (eGFR) was determined using the Collaborative Chronic Kidney Disease Epidemiology (CKD-EPI) eGFR calculation method with the aforementioned clinical chemistry tools. The normal level of creatinine is 0.7–1.2 mg/dl for men and 0.5–1.0 mg/dl for women (Levey AS *et al.* 2009). The calculation of eGFR followed the equation of the variables of sex, age, and serum creatinine levels. The equation formula for calculating eGFR according to the CKD-EPI method for men is 142 × (serum creatinine (mg/dl) /0.9)$^{-0.302}$ x $0.9938^{\text{Age (years)}}$, while for women it is 142 × (serum creatinine (mg/dl) /0.7)$^{-0.241}$ x $0.9938^{\text{Age (years)}}$ x 1.012.

Data analysis

Data analysis was carried out descriptively for characteristics and inferential. This study used multivariate analysis of variables using multiple regression analysis with age, sex, and lipid profile as independent variables and eGFR as the dependent variable. Data were processed using SPSS version 25.0 and analyzed using statistical regression tests. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Respondent characteristics and blood biomarkers

The characteristics of the respondents in this analysis included data on sex, age, blood lipid profile, creatinine level, and blood glucose. An estimated glomerular filtration rate is categorized according to categorical and numerical variables, as presented in Table 1. This study, which started 894 respondents, ended with 893 respondents because one respondent did not meet the inclusion criteria. The respondent was excluded from this study because of incomplete data from blood glucose tests (Fasting Plasma Glucose (FPG) test and Oral Glucose Tolerance Test (OGTT) or 2-hour postprandial glucose test).

The respondents in this study were people who did not have diabetes or prediabetes and were between the ages of 28 and 75 years (with a mean age of 47 years). The average cholesterol, HDL, LDL, and triglyceride levels were 196.75, 48.71, 123.37, and 109.56 mg/dl, respectively. The average creatinine level of the respondents was 0.77 mg/dl. Meanwhile, the average blood glucose levels of the respondents were 89.62 mg/dl (FPG) and 107.19 mg/dl (2-hour postprandial glucose) (Table 1).

The categorization of lipid profile, creatinine and blood glucose levels, and glomerular filtration rate of the respondents is described in Table 2. There were more than 50% of the respondents had normal total cholesterol and only 10% had a high level of cholesterol. High HDL levels were found in more than 50% of the respondents in this sttudy. High LDL levels were found in 10% of the total respondents, but more than 30% of the respondents had LDL levels above normal limits. Less than 6% of the respondents had high triglyceride levels and more than 10% had triglyceride levels that exceeded normal levels. However, the majority of the respondents, more than 80%, had normal triglyceride levels.

The results of this research show that more than 50% of the non-diabetic respondents had relatively good lipid profiles, characterized by more than 50% of the respondents having normal cholesterol levels, high HDL levels, and normal triglyceride levels. Meanwhile, more than 50% of respondents had normal and near-normal levels of LDL. In addition, this study also found that less than 50% of a total of 893 non-diabetic respondents had dyslipidemia.

The eGFR values of the non-diabetic respondent in the abnormal category with the category of mild to severe loss and failure or close failure was 1.8 % of respondents and it can be concluded that the majority, more than 98% of all respondents have a normal eGFR. This study showed that, on average, the lipid profiles of the respondents were good, similarly, the eGFR of the respondents was also mostly normal.

This result of this study was similar to that of another study. A hospital-based case study concluded that dyslipidemia is common in non-diabetic patients with CKD. Dyslipidemia was found in three out of four patients with CKD, with high levels of LDL and low levels of HDL. Lipid parameters and disease development have a high degree of association statistically significant for cholesterol, triglyceride, and HDL levels (Lahane *et al.* 2022). Some studies have concluded that dyslipidemia is possibly a risk factor for kidney disease. Atherosclerosis risk associated with kidney disease in the Community Study found that an increased risk of developing

Table 1. Characteristics of respondents by sex, age, blood lipid profile, creatinine level, blood glucose, and estimated glomerular filtration rate

Variable	n (893)	%	Mean±SD	Min.	Max.
Sex					
Male	276	30.9			
Female	617	69.1			
Age (years)					
Young adults (26–35 years)	93	10.4	32.90±1.99	28	35
Middle-age adults (36–55 years)	600	67.2	44.98±5.59	36	55
Old adults/Elderly (>55 years)	200	22.4	62.13±4.75	56	76
Parameters					
Cholesterol (mg/dl)			196.73±34.23	106.00	427.00
HDL (mg/dl)			48.67±10.85	22.00	99.00
LDL (mg/dl)			123.49±29.10	34.00	242.00
Triglyceride (mg/dl)			107.00±58.44	32.00	492.00
Creatinine (mg/dl)			0.77±0.18	0.40	1.70
FPG (mg/dl)			89.62±5.81	53.00	99.00
OGTT or 2-hours postprandial glucose (mg/dl)			107.19±17.98	55.00	139.00
eGFR			98.47±15.50	41.8	130.79

HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; FPG: Fasting Plasma Glucose
OGTT: Oral Glucose Tolerance Test; eFGR: estimated Glomerular Filtration Rate

kidney dysfunction was associated with low HDL cholesterol levels (Reiner *et al.* 2011). This supports the relationship between the results of this study and other studies that a relatively good lipid profile is associated with a low risk of CKD, which was characterized by a relatively high eGFR in the respondents in this study. The finding that distinguished this study from several other studies was the relationship between increased triglycerides and decreased eGFR. This study concluded that there was no significant relationship between triglycerides and eGFR, whereas several studies concluded that high triglyceride levels were associated with eGFR.

The relationship between age, sex, and lipid profile and estimated glomerular filtration rate (eGFR) is shown in Table 3. It was shown that HDL, LDL, and age had a significant relationship with eGFR with p-values of 0.003, 0.028, and <0.001, respectively. Meanwhile, sex and total cholesterol did not have a significant relationship with eGFR in the results of this study. This study also found that triglyceride tended to have a significant relationship with eGFR (p=0.074). The F-test on the resulting multivariate regression showed a significant slope deviation from zero ($p<0.05$) and the fit was R2=0.413. Decreasing HDL levels, increasing LDL levels, and increasing age will decrease eGFR, which means that the risk of kidney dysfunction increases.

This finding is similar to that of other studies that have show a significant relationship between age and eGFR. This study concludes that young people have higher eGFR than older people (Yue *et al.* 2021). Another study showed that the prevalence of Chronic Kidney Disease (CKD), which is associated with decreased eGFR in the general population, increases with age, with a prevalence of 4% at age less than 40 years, and increases to 47% at age 70 years and older (Ravani *et al.* 2019). Several studies have similar finding that in the relationship between lipid profile and eGFR, total cholesterol does not have a significant relationship with eGFR (Palebangan *et al.* 2020; Zhang *et al.* 2019).

Dyslipidemia is an increase in cholesterol levels, Low Density Lipoprotein (LDL) or triglyceride levels, and decreased High Density Lipoprotein (HDL) levels. Moreover, most previous studies investigating the relationship between renal function and lipid profile have focused on specific types of dyslipidemia, such as High-Density Lipoprotein Cholesterol (HDL-C) (Herrington *et al.* 2016) and Low-Density Lipoprotein Cholesterol (LDL-C) (Chen *et al.* 2013).

Table 2. Categories of lipid profile, creatinine, blood sugar, and glomerular filtration rate of respondents

Lipid profile, creatinine, blood sugar, and glomerular filtration rate	n	%
Total cholesterol		
Normal (<200 mg/dl)	492	55.1
Upper limit (200–239 mg/dl)	312	34.9
High (≥240 mg/dl)	89	10.0
HDL		
Low (male <40 mg/dl; female <50 mg/dl))	385	43.11
High (male ≥40 mg/dl; female ≥50 mg/dl)	508	56.89
LDL		
Normal (<100 mg/dl)	187	21.0
Close to normal (100–129 mg/dl)	336	37.6
Upper limit (130–159 mg/dl)	277	31.0
High (160–189 mg/dl)	93	10.4
Triglyceride		
Normal (<150 mg/dl)	740	82.9
High limit (150–199 mg/dl)	101	11.3
High (200–449 mg/dl)	49	5.5
Very High (>449 mg/dl)	3	0.3
Creatinine		
High (male >1.2 mg/dl; female >1.0 mg/dl)	16	1.8
Normal (male 0.7–1.2 mg/dl; female 0.5–1.0 mg/dl)	870	97.4
Low (male <0.7 mg/dl; female <0.5 mg/dl)	7	0.8
Blood glucose		
Fasting blood glucose		
Normal (<100 mg/dl)	893	100
2-Hour glucose		
Normal (<140 mg/dl)	893	100
eGFR		
Normal eGFR (≥60 ml/minute/1.73 m^2)	877	98.2
Abnormal eGFR		
Mild to severe loss (30–59 ml/ minute /1.73 m^2)	4	0.5
Failure or close to failure (<15 ml/ minute /1.73 m^2)	12	1.3

eGFR: estimated Glomerular Filtration Rate; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein

A number of studies have revealed the role of dyslipidemia in the development of CKD. However, the role of blood lipid types in the incidence and development of CKD seems to be contradictory. Several studies have results that contradict those found in this study. A study by Liang *et al.* (2020) concluded that high TC and TG are associated with decreased renal function and possibly an increased incidence of CKD in the general population, although the exact mechanism of how dyslipidemia may cause CKD is still under investigation. However, the current study suggests that an abnormal lipid profile in the blood leads to ectopic accumulation of lipids, which can occur in virtually any cell type, from mesangial cells to podocytes to proximal tubular epithelial cells. High lipid levels lead to mitochondrial damage and may also be more lethal to proximal tubular cells. High cholesterol levels can also cause macrophage infiltration and foam cell formation in the kidney. It is also believed that high levels of TC and LDL contribute to the risk of renal damage and dysfunction, even when TG levels are within the normal range.

Table 3. Relationship of age, sex, and lipid profile with estimated glomerular filtration rate (eGFR)

Variables	n	B	t	*p*
Age	893	-0.932	-22.467	<0.001
Sex	893	-1.418	-0.758	0.449
Total cholesterol	893	0.852	0.944	0.346
High-density lipoprotein	893	2.650	3.022	0.003
Low-density lipoprotein	893	-1.377	-2.197	0.028
Triglyceride	893	-1.479	-1.787	0.074

Another study reported that High-Density Lipoprotein Cholesterol (HDL-C) was positively correlated with eGFR. Total Cholesterol (TC) and triglycerides were not correlated with eGFR. Low levels of HDL-C are an independent risk factor for the development of chronic kidney disease (Zhang *et al.* 2019).

In addition, a study conducted by McMahon *et al.* (2014) showed the accumulation and breakdown of triglyceride products from lipid metabolism in the blood of patients with CKD, where severe atherosclerotic conditions and pro-inflammatory effects on the vasculature in the kidney parenchyma occur. Previous studies have found that triglyceride levels increase and HDL-C levels decrease with decreasing GFR, while total cholesterol and LDL cholesterol levels tend to remain in the normal range (Herrington *et al.* 2016). Another study reported that Low-Density Lipoprotein Cholesterol (LDL-C) did not correlate with eGFR (Zhang *et al.* 2019).

In addition to low HDL and low eGFR, hypertriglyceridemia is also an early feature of renal failure. According to the study by Bhavsar, patients with impaired renal function show increased TG concentrations even though serum creatinine levels are within normal limits. The study mentioned that individuals with renal insufficiency usually show an abnormally elevated serum Triglyceride Level (TGL) after eating fatty foods (postprandial lipemia). An experimental study showed that the accumulation of TGL-rich lipoproteins (Very-Low-Density Lipoprotein (VLDL)), chylomicrons, and their remnants) in individuals with predialysis CKD is primarily due to a decrease in their catabolism. The most important pathophysiological mechanisms underlying the development of hypertriglyceridemia in renal failure are the downregulation of several gene expressions, changes in the composition of lipoprotein particles, and the direct inhibitory effect of various uremic toxins on enzymes involved in lipid metabolism. Besides HDL and TG, LDL is also involved in this process (Patel 2019). Our study has similar results with the study conducted by Bhavsar, that increase in LDL significantly affects the decrease in eGFR, which has the potential to cause CKD.

This study still has limitations. Despite the use of cross-sectional data analysis, other variables that may affect the glomerular filtration rate, such as diet, fluid intake, and physical activity, were not analyzed. It is necessary to perform the multivariate analysis that uses more variables of cohort data to obtain more valid results if these biomedical parameters are to be used as model predictors.

CONCLUSION

Dyslipidemia and respondent age have a significant relationship with the estimated Glomerular Filtration Rate (eGFR) in non-diabetic respondents. A decline in kidney function may also be the result of dyslipidemia condition.

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DECLARATION OF INTERESTS

The author(s) declare that there is no conflict of interest.

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Dietary Diversity, Stunting, and the Impact of an Education Program on Parents' Knowledge and Attitudes in West Sumba, Indonesia

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ABSTRACT

This pre- and post-test quasi-experimental study aimed to assess dietary diversity and its association with stunting among toddlers (13–60 months), examine the levels of knowledge and attitudes towards balanced and diverse diets among parents or caregivers, and evaluate the impact of customized flashcards and meal boxes as educational tools on parents'/caregivers' knowledge and attitudes in three selected villages in West Sumba, Indonesia. A total of 59 parents/caregivers were recruited, 30% of whom had stunted toddlers. The levels of parents'/caregivers' knowledge and attitudes were moderate. The results from the pre-test study show that Dietary Diversity Score (DDS) did not correlate with stunting status. Furthermore, neither knowledge nor attitudes was discovered to be related to DDS. The use of flashcards and meal boxes in the educational program improved the scores of knowledge ($p<0.05$) and attitudes ($p<0.05$) from the pre-test scores. There was a positive correlation between knowledge and attitudes, either before or after the education program ($r=0.362$, $p<0.05$ and $r=0.562$, $p<0.05$, respectively). To summarize, this study has the potential to be applied in other rural and remote areas.

Keywords: attitude, dietary diversity, education, stunting, West Sumba

INTRODUCTION

Malnutrition can influence nutritional status differently for each individual (Beer *et al.* 2015). Paediatric malnutrition, also known as undernutrition, is a condition where cumulative deficiencies of energy, protein, and micronutrients are caused by a negative balance between nutrient intake and requirement. This condition imparts a detrimental impact on the human body's growth and development (Mehta *et al.* 2013). It was reported that malnutrition is related to illnesses (due to diseases or injuries), environmental factors, and social-behavioural factors (Beer *et al.* 2015). The World Health Organization (WHO) reported that in 2016, about 23% (~154.8 million) of children under five years old suffered from stunting as observed in low height-for-age z scores (below –2 standard deviations from the median height for age of reference population). Stunting in children occurs in the first 1,000 days after birth and is strongly correlated with socioeconomic status, diet, maternal nutritional status, infectious diseases, and micronutrient deficiencies (including suboptimal infant feeding practices) (Raiten & Bremer 2020). This has threatened most developing countries with the major issue of intrauterine growth retardation, particularly maternal undernutrition. Parental education has been initiated to reduce the risk of stunting in developing countries. However, the limited accessibility to execute parental education, especially in remote areas with high stunting rates, is a great hindrance in solving the issue.

Although Indonesia's stunting rate has improved, 27.7% of children under five in Indonesia are still suffering from it. East Nusa Tenggara Province is considered to be one of the provinces with the highest stunting rates in

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Indonesia (38.7%). Stunting among children in East Nusa Tenggara is not only due to the lack of nutrient intake but also due to the lack of water access, climate, geographic condition, and food availability, especially protein. Culture and tradition that prioritize pride over children's nutrition also worsen this condition. Efforts to tackle stunting cannot rely on government or healthcare unit support alone. Parental education also has a significant role in suppressing the stunting rate. Mothers' role is highly linked to all the root causes of stunting and to children's nutritional status. Maternal education could reduce children's malnutrition by up to 43% in developing countries during the 1970s–1990s (Smith & Haddad 2000).

Parental education has been considered to be an effective tool and a suitable delivery strategy to prevent stunting in children. Maternal education is used as an indicator to measure the prevalence level of women with new-borns who have not completed high school (Yue *et al.* 2016). This indicator is important in children's language, cognitive, and academic development. Thus, it can be incorporated with nutritional education as an effective approach to prevent stunting. Most reports suggest that a mother's education is recognized as a fixed parameter regardless of whether many mothers (who are economically and educationally limited) attend school after their children's births (De Silva & Sumarto 2018). For instance, in Indonesia, it was reported in some studies that higher levels of maternal education reduced malnutrition levels (maternal and child double burden) in rural areas (Khaliq *et al.* 2022) and some cities in Indonesia (Mahmudiono *et al.* 2016; Mitra *et al.* 2020). Those studies highlight the importance of nutrition education among parents or caregivers to improve their awareness and knowledge, and in the long run, their attitudes and behaviours.

Therefore, this study aimed to: 1) assess dietary diversity and its association with stunting among toddlers (13–60 months); 2) examine the levels of knowledge and attitudes towards balanced and diverse diets among parents or caregivers, and; 3) evaluate the implementation of flashcards and meal boxes as education tools in improving knowledge and attitudes in three villages in West Sumba, Indonesia—Weipala, Hobajangi, and Praimanakah—through an education program named "*Ibu Bisa*". The "*Ibu Bisa* (literal meaning: mothers can do)" program was committed to providing education for parents or caregivers with under-five children in stunting-prone Indonesian areas.

METHODS

Design, location, and time

The study was conducted using a pre-post interventional study design in three villages, namely, Weipala, Hobajangi, and Praimanakah, in West Sumba. Data collection was carried out from November to December, 2021. Ethical approval was obtained from the Health Research Ethics Committee, Faculty of Public Health, Diponegoro University, Indonesia for this study with approval number 380/EA/KEPK-FKM/2021.

Sampling

The sampling technique used in this research was the purposive sampling technique with Slovin's formula to determine the minimum sample size required to estimate the results for the entire population (n=344), with an 88% precision level. Initially, 72 parents/caregivers with children under five were contacted and participated in this educational program. The respondents were purposively selected based on the Sumba Foundation's target group with eligibility criteria as follows: parents/caregivers who: (1) had children aged 13–60 months; (2) had introduced the children to solid foods; (3) prepared the solid foods for the children's consumption at home, and; (4) had complete anthropometric data of the children. However, ten respondents were excluded as their children were less than 12 months old, and three more respondents were excluded due to incomplete body length data. A total of 59 respondents were included in the final analysis. The exclusion process was performed to ensure that younger child age and incomplete data would not bring any potential risk of confounding factors. Informed consent was obtained, and confidentially was assured.

Children's stunting status measurement was carried out during the intervention based on secondary data (the measurement was carried out monthly by nutritionists of the Sumba Foundation). Body height was measured using a dressmaker's measuring tape during the intervention as it was not possible to use a standardized tool, and the data was collected in a short period. Meanwhile, the Sumba Foundation was using a staturemeter for their monthly measurement. Each parent was asked to report their child's sex and date of birth to ensure a correct calculation of the child's age. A child's stunting status was categorized based on a height/length-for-age z-score according to the WHO's standard growth chart (WHO 2006). A Height/Length-for-Age Z-Score (HAZ) compares a child's height to the height of another child of the same length/height and sex. Based on HAZ, a child was classified as severely stunted (HAZ -3.00 or below), mildly stunted (HAZ between -3.00 and -2.01), normal (HAZ between -2.00 and 1.01), or tall (HAZ 1.01 or above).

Intervention tools and nutrition education

The intervention tools used consisted of flashcards and children's meal boxes (without food). The flashcards contain information about balanced nutrition according to the Ministry of Health of the Republic of Indonesia (MoH RI 2014). There were three selected topics for the flashcards: (1) nutrients and food sources; (2) health problems and imbalanced nutrients; and (3) the importance of physical activity and drinking adequate water. The flashcards were intended to give information about a balanced diet and food diversity to parents/caregivers. Meanwhile, each meal box has three compartments to represent a balanced nutrition guideline named "*Isi Piringku*" or My Plate for children. The purpose of the meal box introduction was to encourage parents/caregivers to practice what they learned through the flashcards and nutrition education to the children. Each parent/caregiver was expected to use one of the meal boxes in preparing a meal for their child by placing a food item in one of the three compartments of the meal box. The meal boxes were made from a silicon material (BPA-free) 7.25 x 2.25 inches in size. One meal box was distributed to one child (in this case, also to one household) and used as an educational tool about portioning size, meal plans, and food group variety.

The intervention process consisted of gathering the respondents in the village meeting hall of each village, introducing the project team and facilitators, administering a pre-test questionnaire session (30–40 minutes), measuring children's anthropometrics, distributing meal boxes and flashcards, running a nutrition education workshop (30–40 minutes), holding a discussion session (5–10 minutes), administering a post-test questionnaire session (30–40 minutes), and closing. The nutrition education was delivered by a certified nutritionist using flashcards and meal boxes through a half-day session (mini-seminar), with a flipchart aligned with the flashcard content as a supporting tool.

Data collection

Pre-test and post-test were carried out to measure the effectiveness of the nutrition tools used in this study using questionnaires. The researcher performed the intervention and data collection with pre-test questionnaires. The questionnaires were written in Indonesian and delivered with the assistance of the Sumba Foundation's local staff. The questionnaires were developed, and the validity and reliability of the questionnaires were tested (n=30). The questionnaires were used to assess knowledge and attitudes based on the balanced nutrition guidelines from the Ministry of Health Republic of Indonesia. Based on Obilor & Amadi 2018, the questionnaires were considered valid if the Pearson correlation is within the range of -1< , r<1, and the value of sig. (2-tailed) is less than 0.05 (based on the analysis results, the Pearson's significance values for the knowledge and attitudes questionnaires were 0.000 to 0.031 and 0.000 to 0.028, respectively) and highly reliable (the Cronbach's alpha values for the knowledge and attitudes questionnaires were 0.665 and 0.810, respectively) (Barbosa 2021).

The questionnaire on nutrition knowledge contains eight questions in the true or false format. Correct answers were converted into scores from 1 to 10, and the final score was classified into bad (<6 points), standard (6–8 points), or good (>8 points) (Yasmin & Madanijah 2010). The questionnaire on nutrition attitudes contain nine questions and uses Likert scales consisting of strongly disagree, disagree, agree, and strongly agree responses. The scoring was conducted using scores from 0 (very inappropriate) to 3 (very appropriate). The total score was classified into bad (<15.00 points with <56% of respondents showing appropriate attitudes), standard (15.00–20.49 points with 56–75% of respondents showing appropriate attitudes), or good (20.50–27.00 points with 76–100% of respondents showing appropriate attitudes) (Arikunto 2010).

Following the pre-intervention, calculation of dietary diversity was conducted retrospectively using a 1-week Food Frequency Questionnaire (FFQ) to generate Dietary Diversity Scores (DDS) as a measure of children's diet quality (Nachvak SM *et al.* 2017). The FFQ was used due to considerations of time restriction and shortage of trained surveyors. The parent/caregiver respondents supplied the dietary intake frequency information of the children. Seven food groups were collected and categorized based on the WHO's infant and young child feeding guidelines (WHO *et al.* 2010), namely: 1) grains, roots, and tubers; 2) legumes and nuts; 3) dairy products (milk, yogurt, and cheese); 4) fleshy foods (meat, fish, poultry, and liver/innards); 5) eggs; 6) vitamin-A-rich fruits and vegetables, and; 7) other fruits and vegetables. If a child consumed at least one food item from a food group during the last week, the group was assigned a score of one (1) for that child, and a score of zero (0) was assigned if none of the food items from the group was consumed. The group scores were then summed up to obtain a dietary diversity score, which ranges from zero to seven, where zero represents non-consumption of any of the food items in the food groups and seven represents the highest level of diet diversification. Minimum dietary diversity was calculated, and the dietary diversity was classified as sufficient (≥4 food groups) or insufficient (<4 food groups).

Data analysis

The Pearson correlation test was used to analyze the questionnaires' validity and the association between knowledge, attitudes, and DDS. The Cronbach's alpha test was used to evaluate the reliability of the questionnaires. The Wilcoxon signed-rank test was used to analyze changes in knowledge and attitudes between pre-intervention and post-intervention. The Spearman's rank correlation test was used to determine the correlation between dietary diversity and nutrition status (stunting). The dietary diversity score was captured as a continuous variable from the count of the number of food groups a child consumed before the intervention program, while nutrition status was captured as a categorical variable. Results were considered significant if $p<0.05$. All analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 21.

RESULTS AND DISCUSSION

The demographic characteristics of parents or caregivers and their children (13–60 months) are shown in Table 1. A total of 59 parents or caregivers were involved in this study with ages ranging from 13 to 60 years old. The majority of them were poorly educated, with only 5% completing college or university education. A total of 15% of the parents or caregivers never even started school. The majority of the parents or caregivers were low monthly earners (under IDR 1,000,000) and unemployed (i.e., being housewives). Meanwhile, the gender-based demographic data of under-five children (13–0 months old) show equal proportions between boys (51%) and girls (49%). The nutritional status of the children was assessed using the height-for-age parameter. Although the data show that the majority of the under-five children were normal/healthy, this study emphasizes that around 30% of all the under-five children were severely and mildly stunted.

Association between dietary diversity and stunting

Several studies suggest that a low-quality, monotonous eating habit is one of the possible risk factors for stunting (Ruel 2003; Hadi *et al.* 2022) because no single meal contains all the necessary nutrients. Table 2 presents the association between DDS and stunting among children under five. The majority of parents or caregivers (98.31%) provided a sufficient variety of diets (≥4 food groups) for their children. According to UNICEF (2012), dietary diversity is an excellent tool to assess the quality of child feeding. It ensures that children receive a variety of nutrients, both macro- and micronutrients. Meeting the suggested minimum dietary diversity can ensure that children get enough energy, protein, and other micronutrients like vitamin A, vitamin D, and iron.

The results of the correlation analysis conducted with the Spearman's rank correlation test show no significant correlation between consumption of a diverse diet and the stunting status among all respondents ($r=-0.096$, $p>0.05$), stunted toddlers ($r=-0.108$, $p>0.05$) and non-stunted toddlers ($r=0.059$, $p>0.05$). Despite the insignificance of this relationship, the results still show a negative direction among stunted children.

Table 1. Socio-demographic characteristics of respondents

Characteristics	n (%)
Parents or caregivers' demographic	
Age (years)	
Young adults (17–30)	26 (44.07)
Middle-aged-adults (31–45)	20 (33.90)
Old-aged adults (above 45)	6 (10.17)
Unspecified	6 (10.17)
Relationship with children	
Father	3 (5.08)
Mother	51 (86.44)
Other family relatives	5 (8.47)
Education level	
Not literate	9 (15.25)
Elementary school	26 (44.07)
Middle school	7 (11.86)
High school	14 (23.73)
College or University	3 (5.08)
Monthly Income (IDR)	
<1,000,000	52 (88.14)
1,000,000–2,000,000	3 (5.08)
2,000,000–3,000,000	3 (5.08)
3,000,000–4,000,000	0 (0.00)
>4,000,000	1 (1.69)
Occupation	
Unemployed (or housewife)	47 (79.66)
Labour	8 (13.56)
Private sector employee	2 (3.39)
Unspecified	2 (3.39)
Children's demographic and nutrition status	
Age	
13–23 months	11 (18.64)
2–5 years	48 (81.36)
Gender	
Boys	30 (50.85)
Girls	29 (49.15)
Nutrition status (based on HAZ)	
Severely stunting	15 (25.42)
Mild stunting	3 (5.08)
Normal	40 (67.80)
Tall	1 (1.69)

Stunted based on height-for-age Zscore (HAZ)
Severely stunting: HAZ<-3.00; Mild stunting -3.00≤HAZ< -2.01; Normal: -2.01≤ HAZ<1.01; Tall HAZ>1.01 SD
IDR: Indonesian rupiah

Such an insignificant relationship between DDS and stunting might be caused by the similar types of foods consumed by the two groups although the eating frequencies and total food intake levels might be different.

Although most of the respondents had already met the minimum dietary diversity score, it remains unclear why West Sumba has such a high prevalence of stunted children under the age of five. A further analysis was performed to assess the proportion of children in terms of food groups consumed before the education program. Table 3 shows that no toddlers consumed milk or dairy products daily. Milk is a good source of proteins, omega-3, vitamins, and minerals, which are important to reduce the risk of stunting (Sjarif *et al.* 2019). The majority of the toddlers (i.e., the toddlers of ≥60% of total respondents) consumed rice and cassava as staple foods (i.e., sources of carbohydrates). Plant-based proteins were mostly consumed from mung beans, tofu, and Indonesian tempeh, while animal-based proteins were mostly consumed from eggs and chicken. Mung beans are highly consumed since they are among the crops that grow easily in West Sumba. Only 46% of the toddlers consumed fish. The majority of the children consumed moringa leaves (*Moringa oleifera*) to reduce stunting (Putra *et al.* 2021), but they still had a limited access to fruit and vegetable choices. Moringa leaves were readily available and growing wildly in their surrounding.

Parents' or caregiver's knowledge and attitudes

Balanced nutrition knowledge is the level of information the parents or caregivers possessed about the balanced diet for toddlers based on the "*Isi Piringku*" or My Plate guideline from the Ministry of Health of the Republic of Indonesia and the functions of macronutrients and micronutrients to support children's development, especially in preventing stunting. The lowest score for overall parents or caregivers during pre-intervention was 2.5, the highest was 10.00, and the mean was 6.11±1.92 (moderate level). After the education program intervention, the lowest knowledge score achieved was 1.25, the highest was 10.00, and the mean was 7.18±1.83 (moderate level) (Table 4). Individual socio-demographic characteristics, including age and education level, were possible factors affecting their learning process. The higher the level of

Table 2. Bivariate correlation analysis of the association between dietary diversity and stunting

	All respondents (n=59)			Stunted toddlers (HAZ<-2.01 SD; n=18)			Non-stunted toddlers (HAZ≥2.01 SD; n=41)		
DDS	n (%)	r	*p*	n (%)	r	*p*	n (%)	r	*p*
0	0 (0.00)	-0.096	0.469	0 (0.00)	-0.108	0.668	0 (0.00)	0.059	0.715
1	0 (0.00)			0 (0.00)			0 (0.00)		
2	0 (0.00)			0 (0.00)			0 (0.00)		
3	1 (1.69)			0 (0.00)			1 (1.69)		
4	0 (0.00)			0 (0.00)			0 (0.00)		
5	5 (8.47)			1 (1.69)			4 (6.78)		
6	53 (89.83)			17 (28.81)			36 (61.02)		
7	0 (0.00)			0 (0.00)			0 (0.00)		
Minimum dietary diversity									
Yes (≥4 food groups)	58 (98.31)			18 (30.51)			40 (67.80)		
No (<4 food groups)	1 (1.69)			0 (0.00)			1 (1.69)		

*$p<0.05$; **$p<0.01$; ***$p<0.001$ using Spearman's Rank Correlation at 95% confidence level; n: number of respondents
HAZ: Height-for-Age Z-score; DDS: Dietary Diversity Score

schooling a person has, Septamarini *et al.* (2019) claims, the more information he/she will learn, increasing his/her level of knowledge. Age will also impact a person's capacity for understanding and perspective on the information presented to him/her (Yuwanti *et al.* 2021). In this study, there were a broad variety of education levels and ages among the respondents.

In addition, this study also measured the parents' or caregivers' attitudes regarding the choice of varied food sources based on balanced nutrition for toddlers. The lowest attitude score during pre-intervention was 9.00, the highest was 27.00, and the mean was 17.34±4.21 (moderate level). After the education program intervention, the lowest attitude score achieved was 12.00, the highest was 27.00, and the mean was 18.75±4.26 (moderate level). There was a significant improvement in both the mean score of knowledge (difference: 1.07±1.77 point with $p<0.05$) and the mean score of attitudes (difference: 1.41±4.32 point with $p<0.05$) after the intervention program.

Knowledge in the context of nutrition refers to the awareness of practices and concepts related to nutrition and health, such as adequate food intake, diet-related diseases, foods representing major sources of nutrients, and dietary guidelines and recommendations (Spronk *et al.* 2014; Ong *et al.* 2021). Good knowledge is the foundation of a good attitude. In our opinion, adequate knowledge and a positive attitude towards a balanced and diverse diet can encourage parents or caregivers to provide foods in terms of both quantity and quality to affect the food consumption of their children.

The association between knowledge, attitudes, and dietary diversity

The relationship between parents' or caregivers' knowledge, attitudes, and dietary diversity is shown in Table 5. There was a low positive correlation between knowledge and attitudes during the pre-intervention ($r=0.362$, $p<0.05$). After the education program, the strength of the correlation between knowledge

Table 3. Proportion (%) of toddlers in terms of their food consumption

Category	Food item	% of toddlers consumed food item
Grains roots and tubers	Rice	92
	Cassava	83
	Corn	56
	Sweet potato	7
Legumes and nuts	Mung beans	97
	Tofu	86
	Tempeh	81
	Soybeans	12
Dairy product	Dairy product	0
Flesh foods	Chicken	90
	Fish	46
	Pork	24
	Buffalo	12
	Beef	8
Eggs	Eggs	97
Vitamin a rich fruits and vegetables	Papaya	97
	Moringa leaves	95
	Cassava leaves	80
	Papaya leaf	76
	Water spinach	54
	Spinach	51
	Chinese cabbage	24
	Mango	24
	Watermelon	2
Other fruits and vegetables	Banana fruit	93
	Tomatoes	51
	Banana blossom	29
	Eggplant	29
	Yardlong bean	25
	Papaya flower	20
	Chayote	15
	Cucumber	14
	Pineapple	10
	Cabbage	3
	Jackfruit	3

and attitudes increased ($r=0.562$, $p<0.05$). Finally, there was no significant correlation both between knowledge and DDS and between attitudes and DDS during the pre-intervention period.

Knowledge is substantial for parents to provide sufficient care to their children. A previous study reported that knowledgeable mothers will provide more nutritious foods for their children and thus can maintain their children's nutritional status based on HAZ (Simanjuntak *et al.* 2019). Attitude is one of the factors that can encourage one to take certain actions. Parents' or caregivers' knowledge and attitude levels regarding a balanced diet based on the "*Isi Piringku*" or My Plate guideline for toddlers improved significantly after the education program. The improvement of knowledge was followed by the improvement of attitudes. Therefore, the application of educational flashcards and toddler meal boxes also significantly strengthened the positive correlation between parents' or caregiver's knowledge and attitudes.

The parent's or caregivers' practice in providing a variety of foods for their children prior to the intervention program was analyzed. Although there were no significant correlations between DDS and knowledge ($r=0.021$, $p>0.05$) and between DDS and attitudes ($r=-0.011$, $p>0.05$), dietary diversity was found to be a potential protective factor against stunting. The negative correlation between DDS and stunting was observed in all respondents and stunted toddlers ($p>0.05$), but it was negligible among non-stunted toddlers. Moreover, there were several studies reporting the importance of dietary diversity to prevent stunting among children under five (Khamis *et al.* 2019; Rah *et al.* 2010; Wali *et al.* 2020).

CONCLUSION

In sum, over 98% of the parents or caregivers involved in this study had provided at least four food groups. However, none of the toddlers consumed milk and dairy products. There was no correlation between dietary diversity and stunting status among all respondents ($r= -0.096$, $p>0.05$), stunted children ($r=-0.108$, $p>0.05$), and non-stunted children ($r=0.059$, $p>0.05$) at a 95% confidence level. The results of the examination of parent's/caregivers' levels of knowledge and attitudes towards a balanced and diverse diet show that, on average, the parents'/caregivers;

Table 4. Distribution of respondent based on knowledge and attitude category

Variable		Category	n (%)	Mean±SD	Lowest score	Highest score	Change score	
							Mean±SD	*p*
Knowledge level[1]	Pre-intervention	Low	20 (33.90)	6.11±1.92	2.50	10.00	1.07±1.77	0.000***
		Moderate	30 (50.85)					
		High	9 (15.25)					
	Post-intervention	Low	13 (22.03)	7.18±1.83	1.25	10.00		
		Moderate	26 (44.07)					
		High	20 (33.90)					
Attitude level[2]	Pre-intervention	Low	18 (30.51)	17.34±4.21	9.00	27.00	1.41±4.32	0.029*
		Moderate	28 (47.46)					
		High	13 (22.03)					
	Post-intervention	Low	12 (20.34)	18.75±4.26	12.00	27.00		
		Moderate	29 (49.15)					
		High	18 (30.51)					

[1]Knowledge category based on total score (Low: <6; Moderate: 6–8; High: >8)
[2]Attitude category based on total score (Low: Less than or equal to 14.59; Moderate: Between 15.00–20.49; High: More than or equal to 20.5)
n: number of respondents; SD: Standard Deviation
*p<0.05; **p<0.01; ***p<0.001 using Wilcoxon signed rank test at 95% confidence level

Table 5. Pearson's correlation coefficient analysis between knowledge, attitude, and dietary diversity attributes

Variable (metric)	Pearson's r	*p*
Pre-Intervention		
Knowledge–Attitude	0.362**	0.005
Knowledge–Dietary diversity	0.021	0.872
Attitude–Dietary diversity	-0.011	0.933
Post-Intervention		
Knowledge–Attitude	0.562***	<0.000

*p<0.05; **p<0.01; ***p<0.001 using pearson correlation at 95% confidence level

knowledge and attitudes were at moderate levels. The application of educational flashcards and toddler meal boxes could significantly improve parents' or caregivers' knowledge (p<0.05) and attitudes (p<0.05) in rural areas. These intervention tools increased not only the levels of knowledge and attitudes but also the strength of the positive correlation between two parameters (pre-test vs post-test: r=0.362, p<0.05 vs r=0.562, p<0.05).

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DECLARATION OF INTERESTS

The authors have no conflict of interest.

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Risk Factors Associated with Underweight Children Under the Age of Five in Putrajaya, Malaysia: A Case-Control Study

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ABSTRACT

The study aimed to determine the associated factors for underweight among children under five years old in Putrajaya, Malaysia. This was a case-control study with a one-to-one ratio matched by sex as well as by three age categories (6–11 months, 12–35 months, dan 36–59 months) between underweight and normal-weight children. There were 364 underweight children and 364 children with normal weight recruited from four government clinics and 118 preschools in Putrajaya. Both groups were assessed via face-to-face interviews; anthropometric measurements; haemoglobin level through finger prick blood sample; and a self-administered 3-day food diary. Underweight is defined as a weight-for-age z score less than -2SD based on World Health Organization (WHO) 2006 Growth Chart. The logistic regression's final model revealed that various factors were significantly associated with underweight among children under five in Putrajaya. These factors included father being employed as a non-government servant [aOR:1.45 (95% CI:1.04–2.02) compared to government servant], children from B40 group with a monthly household income less than <RM 7,380 (USD 1727.33) [aOR:2.17 (95% CI:1.01–4.66) compared to T20], monthly expenditure for childcare less than RM 1,000 (USD 234.06), [aOR:1.77 (95% CI:1.01–3.10) compared to ≥RM 2,000], underweight mother during prepregnancy [aOR:1.89 (95% CI:1.10–3.26)] compared to normal weight, anemic children [aOR:1.57 (95% CI:1.15–2.16)] compared to normal children, children using pacifiers [aOR:1.75 (95% CI:1.21–2.73)] compared to not using pacifiers and children staying with unregistered babysitters [aOR:2.33 (85% CI:1.52–3.59)] compared to those attending kindergarten. The above findings suggest several factors are significantly associated with underweight among children under five years old. Therefore, it highlights on the importance of improving household socioeconomic status, maternal nutritional status, and infant and young child feeding practices to prevent underweight issues in this population.

Keywords: below five years, children, undernutrition, underweight, urban

INTRODUCTION

Malnutrition comprises undernutrition, micronutrient malnutrition, and obesity (World Health Organization (WHO) 2017). Undernutrition is the state when an individual lacks an adequate amount of nutrients in his or her body. According to a previous study, around 45% of deaths in children under the age of five are caused by malnutrition, and emerging nations have the highest frequency of early childhood malnutrition based on recent rapidly economic growth (Guyatt *et al.* 2020). Presence of underweight in early childhood may have

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long-lasting implications. This condition to some extent, interferes with children's physical and cognitive development and reduces their academic performance (Akombi *et al.* 2017). Underweight in children might indicate a nutritional problem that reflects in both wasting and stunting issues (Kurnianingtyas *et al.* 2021). This state of childhood malnutrition could be due to insufficient intake of one or more specific nutrients such as vitamins or minerals (Wells 2019). Nutritional status of women and children are influenced by multiple socioeconomic and cultural factors (Hossain *et al.* 2020; Li *et al.* 2020).

The United Nations (U.N.) approved 17 Sustainable Development Goals (SDGs) in 2015 in order to address the concerns of poverty, inequality, and climate change in the twenty-first century. In favour of improving nutrition, the target is to end malnutrition by 2030 (U.N 2015). The worldwide incidence of underweight among children under the age of five was 15% in 2013 (Mgongo *et al.* 2017). Children with Low Birth Weight (LBW) tend to become underweight later in life if their nutrition intake is not being supervised suitably (UNICEF 2019). Therefore, in Malaysia, an action plan to monitor children under five years old with underweight status was incorporated in the National Plan of action for Nutrition of Malaysia III (2016–2025) (MoHM 2016).

There was 13.7% of children under five years old in Malaysia were underweight (IPH 2016). In comparison, a study of 3,600 children aged 16 years from 14 impoverished communities in Peninsular Malaysia discovered that 32.6% of boys and 35.9% of girls were underweight. Children's nutritional status were affected by different factors such as parental education, feeding practises, maternal nutritional state, the number of under-five children in one household, poverty, access to health facilities, and disparities between urban and rural areas (Motbainor *et al.* 2015). Malnutrition may occur when one receives an inadequate intake of energy and nutrients which are required for normal growth.

The National Health and Morbidity Survey (NHMS) 2016 has shown that Putrajaya is the fourth state with higher stunting cases after Terengganu, Kelantan, and Pahang (IPH 2016). Despite the fact that the Federal Territory of Putrajaya houses the majority of Malaysia's government officials and has some of the best facilities in the nation, the level of nutritional status of the children residing there was queried. Putrajaya serves as the country's administrative centre. Given this particular reason, and due to lack of data, the study was determined to identify the factors associated with underweight status among children under five years old in Putrajaya.

METHOD

Design, location, and time

The survey was a case-control study over a one-to-one ratio matched by sex and three categories by age (6–11 months, 12–35 months, and 36–59 months), carried out in finding the associated factors of underweight in Putrajaya (Ahmad *et al.* 2021). The study took place in Putrajaya beginning from September 2018 until January 2019.

Sampling

Inclusion and exclusion criteria. The inclusion criteria were children aged 6 to 59 months old, Malaysian citizens, and a Putrajaya resident for no less than 6 months. The children with a mental or physical disability or ill during data collection and children with chronic diseases were excluded from joining the study.

Sample size estimation. The sample size was estimated based on the formula for comparing 2-Proportions (using PS software) according to an identified risk factor to underweight in NHMS 2016 data (maternal underweight) with type 1 error (α) of 0.05, and power (β) of 0.80. From the calculation, the minimum sample size required for each group was 318 (IPH 2016).

Recruitment and implementation. The ethical study approval was obtained from the Medical Research Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-18-847-41455). Parental informed consent was obtained prior to the data collection. The case group consisted of children under five years with underweight (weight-for-age <-2SDs), and the control group consisted of children within the normal range for all growth indicators (weight-for-age, height-for-age, weight-for-height/length, and BMI-for-age between -2SDs to +2SDs z score from the median). Screening for recruitment based on eligibility was done during Phase I (screening; 12th September 2018 to 12th October 2018) in preschools and all government health facilities in

Putrajaya. During Phase II, trained nutritionists did the face-to-face interviews among caregivers between 16th October to 31st January 2019 to obtain information from caregivers/parents.

Data collection

Questionnaire. The tablet questionnaire contains seven modules that ask about sociodemographic and socioeconomic characteristics, the respondent's and his or her mother's health and medical record, knowledge and practise of parents or carers regarding child feeding, dietary behaviour of the children, Infant and Young Child Feeding (IYCF) history, food security, and screen time/physical exercise. The questions asked in this study were previously verified. The questionnaires were used for face-to-face interview with the respondents.

This set of questionnaires was used for assessing breastfeeding practices, history of breastfeeding, infant feeding and complementary diet. The questionnaire was adapted from WHO Global Consensus Meeting on Indicators of Infant and Young Child Feeding 2007 (WHO 2007). Meanwhile, three days of food intake of children were completed by the parent and caregiver with two days during weekdays and one day during the weekend. The quantities of all food and beverages consumed by the eligible child were recorded by the parent and caregiver in a given 3-days food diary using standard household measurements. For children who went to preschool during weekdays, the food intake at school was recorded by their teacher using a separate food diary form. The data collection team collected the 3-days food diary and separated food diary from preschool and probed during the second visit. The 3-day food diary data, however, is not presented in this paper. A mobile tablet with real-time data entry was utilized for face-to-face interviews to obtain all the relevant information. Data is saved online at the institute's local server in CSV format.

Anthropometric measurements. Tanita Personal scale HD 319 (TANITA Cooperation, Tokyo, Japan) and SECA Stadiometer 213 (SECA Cooperation, Humberg, German) was used to measure the body weight and body height of the children and their parents/caregivers (Baharudin *et al.* 2017). The measurements were rounded to the nearest 0.1 kg for body weight and 0.1 cm for body height. For infants or children who were unable to stand properly, their weight was measured with SECA 354 digital baby scale and their length with SECA 210 mobile baby measuring mat. All measurements were taken twice, and the Survey Creation System (SCS) application recorded the readings. The tools were calibrated daily prior to data collection. For statistical analysis purposes, the study used the average weight and height values. The WHO Anthro Software version 3.2.2 was used to identify the nutritional status of respondents (WHO 2011).

Hemoglobin assessment. Hemoglobin assessment by trained nurses to all consented respondents using a portable HemoCue analyzer (HemoCue® Hb 201). The results of haemoglobin concentration were entered into the mobile device. A haemoglobin level of less than 11.0 g/dl was used to define anaemia in children (Ahmad *et al.* 2015; WHO 2011).

Dietary intake. Dietary intake of the respondents was assessed by the three days food diary. Their parents/caregivers were requested to fill in the food diary for two weekdays and one day over the weekend. Teachers from preschools were requested to record food intake during school time for pre-schoolers. The trained nutritionist performed a quality check of the food diaries prior to data entry.

Data management. Data were submitted to the server located in the Institute of Public Health (IPH) online. The data received by the server in CSV form were converted into SPSS format. Weekly deliveries of the food diaries were made to IPH. The team members in IPH keyed in the food intake of respondents into Nutritionist Pro Software version 7.5 for analysis of calories and nutrient intake.

Data analysis

Descriptive statistics were performed for all variables. Simple logistic regression was performed to determine the association between underweight and all independent variables. Variables with p-value <0.25 (Bursac *et al.* 2008) and clinically significant variables were included in the multiple logistic regression analysis. To provide a preliminary model, multicollinearity and interactions were tested. IBM SPSS Statistics for Windows, version 23.0 was used to perform the statistical analyses.

The three model fit assessments are the Hosmer-Lemeshow test, the Classification table, and the vice Area under Receiver Operating Characteristics (ROC) curve. The independent variables with p-value<0.05 were taken as associated factors of underweight status. Meanwhile, factors with odds ratio >1.00 were considered as risk factors and factors with odds ratio ≥1.00 were regarded as protective factors.

RESULTS AND DISCUSSION

About 1,516 (18.5%) children screened for the study were underweight. Among the underweight children, 845 (19.9%) were boys and 671 (16.9%) were girls. The study successfully recruited a total of 728 children (underweight=364, normal=364). From multiple logistic regression analyses, the preliminary final model found mid-parental height <150 cm, father's occupation in the private sector, B40 household income group, monthly expenditure for childcare less than RM 1,000 (USD 234.06), underweight prepregnancy BMI, having low birth weight, being anaemic children, bottle feeding, using a pacifier and being taken care by babysitter while the parents were at work, were associated with underweight as described in Table 1.

More than half of the children from both case and control groups have parents with mid-parental height of 160 cm and above. Most parents in both case and control groups achieved tertiary education and were employed. However, more than half earned below the bottom 40 (<RM 7,380 (USD 1727.33)). In general, about half of the families spent (<RM 1,000 (USD 234.06)) on childcare. There was a higher proportion of children from the case group (15.7%) whose mothers were underweight during prepregnancy than children from the control group (6.9%).

Table 1. Factors associated with underweight among children under five years old in Putrajaya

Variables	Underweight n (%)	Normal n (%)	Simple logistic regression		Multiple regression	Logistic
			OR (95% CI)	*p*	AOR (95% CI)	*p*
Mid-parental height (cm)						
<150.0	27 (7.4)	11 (3.0)	2.68 (1.29–5.55)	0.008	3.03 (1.38–6.62)	**0.006***
150.0–159.9	141 (38.7)	139 (38.2)	1.11 (0.82–1.50)	0.510	1.01 (0.73–1.40)	0.950
≥160.0	196 (53.8)	214 (58.8)	1		1	
Father's occupation						
Government servant	208 (57.1)	223 (61.3)	1			
Non-gov. servant	154 (42.3)	138 (37.9)	1.20 (0.89–1.61)	0.237	1.45 (1.04–2.02)	**0.050***
Not working	2 (0.5)	3 (0.8)	0.71 (0.12–4.32)	0.714	0.37 (0.04–3.63)	0.392
Household income (monthly)						
B40 (Below 40%)	230 (63.2)	184 (50.5)	2.31 (1.14–4.66)	0.020	2.17 (1.01–4.66)	**0.048***
M40 (Middle 40%)	121 (33.2)	156 (42.9)	1.43 (0.70–2.93)	0.325	1.59 (0.73–3.48)	0.243
T20 (Top 20%)	13 (3.6)	24 (6.6)	1		1	
Monthly expenditure for childcare						
<RM 1,000 (USD 234.06)	200 (54.9)	159 (43.7)	2.21 (1.34–3.65)	0.002	1.77 (1.01–3.10)	0.046*
RM 1,000–RM 1,999 (USD 468.61)	135 (37.1)	154 (42.3)	1.54 (0.92–2.57)	0.097	1.49 (0.85–2.61)	0.112
≥RM 2,000 (USD 468.85)	29 (8.0)	51 (14.0)	1		1	
Pre-pregnancy BMI						
Normal	208 (57.1)	198 (54.4)	1		1	
Underweight	57 (15.7)	25 (6.9)	2.17 (1.30–3.61)	0.003	1.89 (1.10–3.26)	**0.022***
Overweight or obese	99 (27.2)	141 (38.7)	0.67 (0.48–0.92)	0.014	0.72 (0.51–1.102)	0.062

Continue from Table 1

Variables	Underweight n (%)	Normal n (%)	Simple logistic regression		Multiple regression	Logistic
			OR (95% CI)	*p*	AOR (95% CI)	*p*
Birth weight status						
Normal birth weight	286 (78.6)	340 (93.4)	1		1	
Low birth weight	78 (21.4)	24 (6.6)	3.86 (2.38–6.27)	0.001	3.25 (1.89–5.60)	**0.001***
Children Hb status						
Normal	174 (47.8)	213 (58.5)	1		1	
Anaemic	190 (52.2)	151 (41.5)	1.54 (1.15–2.06)	0.004	1.57 (1.15–2.16)	**0.005***
Use of pacifier						
Yes	318 (87.4)	296 (81.3)	1.59 (1.06–2.38)	0.026	1.75 (1.21–2.73)	**0.014***
No	46 (12.6)	68 (18.7)	1		1	
Place of stay						
Kindergarten	233 (64.0)	291 (79.9)	1		1	
Babysitter	92 (25.3)	42 (11.5)	2.74 (1.83–4.10)	0.001	2.33 (1.52–3.59)	**0.001***
Relative	39 (10.7)	31 (8.5)	1.57 (0.95–2.60)	0.078	1.30 (0.74–2.26)	0.357
MVPA time in a day						
<180 minutes	27 (7.4)	22 (6.0)	1.25 (0.70–2.23)	0.460	-	
≥180 minutes	337 (92.6)	342 (94.0)	1		-	

*p<0.05 for logistic regression analysis Hosmer-Lameshow test=0.837 (>0.05); Classification table=63.7%, Area under Receiver Operating Characteristics (ROC) curve=0.712

B40 is household income <RM 7,380 (<USD 1,727.33); M40 is household income between RM 7,380–RM 14,789 (USD 1,727.33–USD 3,461.44); T20 is household income ≥RM 14,790 (≥ USD 3,461.44) ; 1 MYR=0.23USD

AOR: Adjusted Odds Ratio; MVPA: Moderate to Vigorous Physical Activity; OR: Odds Ratio

Note: Variable added but not shown in table (mother's education, mother's occupation, father's education, monthly expenditure for food, utility and transport, mother age during pregnancy, weight gain during pregnancy, complication during pregnancy, number of antenatal visits, child feeding knowledge, practice and behavior, delivery method, delivery status, birth length, birth head circumference, number of siblings, the age gap between elder and younger sibling, frequent illness, injury and worm infection, initiation breastfeeding, breastfeeding status, exclusive breastfeeding, predominant breastfeeding, age stop breastfeeding, formula milk, use of bottle feeding, MDD status, achievement of kcal intake, achievement of protein intake, food insecurity, sleep time, screen time, and MVPA time

There were 13.2% of children from the case group, and 6.2% of children from the control group delivered prematurely. A significantly higher proportion of underweight children were anaemic (52.2%) than normal children (41.5%).

A significantly higher proportion of underweight children (87.4%) were given pacifiers than normal children (81.3%). By childcare, a higher proportion of underweight children was found among those taken care of by babysitters or relatives. Multivariate analysis using logistic regression found that underweight children were associated with; short parental stature, low household income, less expenditure spent for childcare, underweight pre-pregnancy mother, low birth weight, anaemic children, and pacifiers' practice and being taken care of by a babysitter.

In general, there are nine risk factors significantly associated with being underweight among children under five years old. Our study found that Low Birth Weight (LBW) is the strongest risk factor for underweight. The finding from this study was comparable with the previous study conducted in Indonesia. A previous study reported higher proportion of Indonesian children with history of low-birth-weight were underweight compare to their counter part of normal birth weight (Kurnianingtyas *et al.* 2021). Several previous studies consistently reported that LBW is a significantly associated factor in being underweight in urban and rural areas

(Utami *et al.* 2018; Ntenda 2019). On the other hand, a study reported that LBW was particularly caused by maternal health (Mumbare *et al.* 2012).

This study described that the children with short stature parents with a mid-parental height of less than 150 cm were three times more likely to become underweight than children with parents with a mid-parental height of 160 cm and above. This finding can be supported by a similar study from Korea reporting that children with lower mid-parental height also have lower weight (Suh & Kim 2020). However, an earlier study from Sweden reported only a weak association of mid-parental height with the child being underweight (Berglund *et al.* 2016). Nonetheless, mid paternal height showed no significant association with underweight (Amin & Julia 2016). The World Health Organisation Multicentre Growth Reference Study (WHO MGRS) found that in situations with widely varied parental heights, mid-parental height consistently predicted a larger proportion of observed variability in attained child length (Garza *et al.* 2013). The current review on malnutrition and genetics highlights on the need of doing more research on malnutrition genetics to provide better understanding of the genetic risks of malnutrition, which may help identify ideal targets for malnutrition intervention and treatment (Duggal & Petri 2018).

Due to their working mothers, numerous children were placed under the care of babysitters at a young age. This current finding indicates a notable correlation between the babysitters' care and the prevalence of underweight children. This condition might be attributed to babysitters who cared for the babies/children only having lower formal education or lack of training in childcare compared to caregivers in kindergarten. In Malaysia, under the Act of Care Centers 1993 (ACT 506), caregivers must provide healthy, balanced, safe food for all children under their care (MoHM 2018). They must attend continuous training and short courses to provide professional care to the babies/children in kindergartens or nurseries. The caregivers are knowledgeable and experts in their field, understand well and perform nursing-related tasks according to established standards (Department of Social Welfare 2020). Nutrition Division and Department of Social Welfare Malaysia provided a Summary of the Menu Planning Guide at the Care Centre to ensure the quality of meals provided in children's centres (MoHM 2018). This indicates that the complementing purpose of outstanding childcare in preschools and day care institutions is critical in decreasing malnutrition in people with limited resources. A survey conducted in Cyprus found that a higher percentage of children were undernourished when cared for by babysitters. The study also reported that more children that were cared for by babysitters were underweight than those cared for by others. Babysitters may not provide the child with the appropriate nutritional support needed (Mousa 2018).

Furthermore, in our study, children with underweight status were significantly associated with low household income and the least monthly expenditure. An important factor was underlying most of the determinants of underweight children in poverty. This outcome was consistent with earlier research from low- and middle-income nations (Fagbamigbe *et al.* 2020; Kanjilal *et al.* 2010). Generally, individuals residing in poorer households are usually unable to achieve food security (Kimani-Murage 2013) and are less likely to spend money on childcare. Evidence from other studies suggests that low-income status may reduce the accessibility to healthier foods, hence consuming healthy foods (Nackers & Appelhans 2013).

In this current study, low maternal prepregnancy weight was significantly associated with underweight children and was consistent with the previous studies, which determined low maternal prepregnancy BMI on the physical growth of the children (Li *et al.* 2018). The BMI of mothers was considerably high and strongly correlated with the nutritional health of their children, according to a study on 246 mothers in North Bengal, India (Tigga & Sen 2016). Despite that several studies from developed countries were concentrating on the association between high maternal prepregnancy BMI (overweight or obesity) and offspring obesity and intellectual development (Yu *et al.* 2013; Veena *et al.* 2016) study on prepregnancy underweight and associated outcomes are still rarely considered (Li *et al.* 2018). Our findings revealed that gaining ideal weight before pregnancy among women is very important in preventing underweight status among children in order to maintain optimum growth of children (Gul *et al.* 2020).

The use of a pacifier, mostly in young child feeding practice, is identified as a risk for underweight rather than bottle feeding and those who do not achieve Minimum Dietary Diversity (MDD). The previous study reported that pacifier usage among young children would lead to a shorter duration of breastfeeding and reduced food intake (Campos *et al.* 2018). In addition, infants or children exposed to bottles or pacifiers were more likely to have Oral Motor Dysfunction (OMD). This study also revealed a significant association between fathers' employment in the non-government sector and underweight children. Another Korean study could support this finding that reported poor household socioeconomic conditions (household wealth) were the strongest factors in most countries (Li *et al.* 2020). However, we did not find the same association between mothers' employment and underweight children. These results could not be compared to those of an earlier study carried out in Ludhiana, Punjab. The previous study found that most children with normal weight have fathers working in the private sector. Hence a father's occupation affects a child's nutritional status (Kaur *et al.* 2017).

An association between being underweight and anemia was observed in our study. A similar association was seen in a study conducted among children in rural areas of Shaanxi province in northwestern China showed that the prevalence rate of underweight among 118 infants with anemia was 45.3% (Yang *et al.* 2012) while a study done in two areas in Ethiopia: Eastern Oromia (Babble District) and South-Central Tigray (Enderta & Hintalo Wajirat Districts) showed that 66.7% underweight children were anemic (Roba *et al.* 2016).

Additionally, there were a number of issues with this study. There was a lack of diversity in the sociodemographic background of the respondents because their parents were mainly employed in the government sector, and most of them were Malay. This was a case-control study with the design allowing us to look at multiple risk factors at one time. The findings of this study provide necessary facts to stakeholders and policymakers to develop/plan interventions towards reduction in the prevalence of underweight children in Putrajaya.

CONCLUSION

According to our findings, nine factors such as low birth weight, mid parental height less than 150 cm, taken care by a baby sitter, B40 household income, underweight mother, monthly child expenditure for children less than RM 1,000, using of a pacifier, father working in non-government agencies and children with anemia are significantly associated to underweight. These findings may point to potential causal factors that may have an impact on the underweight problem among younger children, such as low socioeconomic status, inadequate prenatal care, a lack of education or knowledge about baby and young child feeding practises. This study and its findings provide stakeholders and policymakers with convincing evidence for taking appropriate measures to address and improve household socioeconomic status; maternal nutritional status; and infant and young child feeding practices in preventing the underweight issues among youngsters especially children under five years old in Putrajaya.

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DECLARATION OF INTERESTS

These authors declare that there is no conflict of interest in any form.

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Predicted Glycaemic Index Values of Rice Prepared with Different Cooking Methods

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ABSTRACT

The current study investigated how the preparation methods impact the nutritional composition and estimated Glycaemic Index (eGI) of the white and brown rice samples. The analysis of proximate and eGI was conducted after the white and brown rice were prepared through these cooking methods: (1) cooking rice without additional coconut oil and not being refrigerated (control); (2) cooking rice without additional coconut oil and being refrigerated (XCOR); (3) cooking rice with additional coconut oil and not being refrigerated (COXR); and (4) cooking rice with additional coconut oil and being refrigerated (COR). The result showed that the COR method recorded the lowest calorie for both white and brown rice (223.93 kcal and 169.90 kcal per 100 g, respectively). Meanwhile, the COR method also recorded the lowest eGI for both white rice (2.31, 6.36, 6.07, 4.55, 3.02, 2.22 nm/min) and brown rice (1.44, 1.92, 1.92, 1.36, 0.66, 0.27 nm/min) at 20, 30, 60, 90, 120, and 180 min, respectively. In conclusion, consuming refrigerated rice that has been cooked with coconut oil can be used as an alternative preparation technique to lower both calorific value and glycaemic index for the preparation of healthier rice meals for health-conscious individuals.

Keywords: brown rice, coconut oil, eGI, refrigeration, white rice

INTRODUCTION

Changing dietary habits represents one of the most influential lifestyle factors. The fundamental reason for obesity and overweight is an energy imbalance between calories consumed and calories expended, which is a positive energy balance involving the total energy intake exceeding the total energy expenditure (World Health Organization (WHO) 2020). Besides, Diabetes Mellitus (DM) is a condition disease where the blood glucose level is surged irregularly due to the failure of the body to generate insulin or to use it to the fullest (Ganjifrockwala *et al.* 2017). It is linked with serious complications such as stroke, ischemic heart disease, cancer and possibly can lead to organ failure (Ng *et al.* 2020). Type 2 Diabetes (T2DM) is one of the common modern lifestyle-linked diseases associated with the high intake of refine carbohydrates and low consumption of Dietary Fibre (DF) (Helmyati *et al.* 2020). Positive energy balance may be due to unhealthy eating habits and an increased intake of energy-dense foods that are high in calories and sugars but low in DF content (Tanaka *et al.* 2020). This may have a significant adverse effect on nutritional status because of the exclusion of adequate quantities of fat, protein, and other essential nutrients.

Most Malaysians consume cooked rice daily as their staple food. White Rice (WR) and Brown Rice (BR) are commonly consumed in Malaysia. BR is a whole grain, containing all parts of the grain: bran, germ, and endosperm, whereas WR had the bran and germ removed (Santos & Timoteo 2016). Thus, BR contains more DF and provides several vitamins and minerals. A large serving of high-calorie dishes containing carbohydrates such as rice, bread, and pasta in the daily diet contributes to excess energy intake and subsequent weight gain. Consuming an excessive amount of carbohydrates, especially with a high glycaemic load, results in a large metabolic load on the body, resulting in an increase in the risk of developing several disorders, including T2DM (Joanne 2018). Due to the excess glucose in the blood, a quick elevation in blood glucose will put a high demand on the beta cells of the pancreas for insulin (Joanne 2018). This extra glucose will then be converted to glycogen, which will be

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deposited as fat in muscles and the liver (Tanaka *et al.* 2020). This condition can cause obesity if the person does not burn the excess calories (Tanaka *et al.* 2020).

Starches are one of the dietary carbohydrates that are made of two different polymers: amylose (essentially linear) and amylopectin (branched) (Arik Kibar *et al.* 2013). Starches can be divided into two types, which are Resistant Starch (RS) and Digestible Starch (DS) (Birt *et al.* 2013). An important fact is that the DS can be converted to the RS. This may be due to the interaction of amylose with other molecules when it is subjected to thermal treatments. The RS can be formed through the retrogradation process, which involves the process of cooling cooked starch after undergoing the cooking process beyond its gelatinisation temperature to form retrograded starch, RS3 (Wang *et al.* 2015). Another way to increase the resistance of starch is by developing RS5, self-assembled starch V-type complexes through the use of coconut oil as an acid combined with heat-moisture treatment during cooking (Arik Kibar *et al.* 2013). RS5 is formed when the hydrocarbon tail of the lipid and the helical cavity of amylose are joined together (Birt *et al.* 2013).

Dietary modifications, including fibre-rich food intake, decreased energy density, and high protein intake, aid in achieving an energy deficit to help weight loss and could be a primary solution to overcome obesity. Involving diet modification such as a low Glycaemic Index (GI) diet by choosing low-GI foods may be more effective than a high-GI diet at reducing body weight, especially in obese people, and controlling glucose and insulin metabolism in T2DM patients. Consuming a low-GI diet causes the glucose to be released slowly and steadily, reducing the high spike in blood sugar, which results in lowering the insulin response and then improving insulin sensitivity (Wang *et al.* 2015). The reduction in insulin secretion gives greater access to using the fat stores to generate energy (Birt *et al.* 2013).

The GI of food can be determined to assess the ability of the carbohydrates it contains to raise Blood Glucose Levels (BGL). Since in vivo GI determination heavily relies on the involvement of healthy human subjects through the consumption of the test food and requires the withdrawal of their blood, the in vitro estimated GI (eGI) assessment is advisable to be performed first. By performing the eGI through an enzymatic reaction approach, the nutritionist or analyst should be able to predict or estimate how fast the carbohydrate (digestibility rate of the starch of the test food in relation to the digestibility of starch in the reference material) raises the BGL. If the test food shows the potential to lower a GI, then the results obtained from eGI can be confirmed or validated further through an accurate in vivo GI study (Demirkesen-Bicak *et al.* 2021). In brief, the eGI is performed to extrapolate the percentage of starch hydrolysed at time t (min) and the equilibrium percentage of starch hydrolysed after 180 min (Eyinla *et al.* 2022).

In line with the fact that DF promotes satiety and satiation, an increase in DF intake was associated with better glycaemic control and more favourable CVD risk factors (Helmyati *et al.* 2021). As an alternative of starch replacement, DS in rice with a high concentration of RS by using coconut oil and refrigeration is proposed because it results in lowering the GI value and reducing energy density due to its lower calorie content, which is 2 kcal/g (Birt *et al.* 2013). Moreover, the presence of oleic acid (unsaturated fatty acid) in coconut oil (Santos *et al.* 2020) was found to be effective in decreasing starch digestibility by forming an amylose-lipid complex and making it resistant to enzymatic breakdown (Birt *et al.* 2013). Since the rate of starch digestibility of the test food is influenced by the presence of DF and RS, the current study emphasises how the preparation methods of rice impact the Estimated Glycaemic Index (eGI). The study aims to investigate the nutritional composition and predict the eGI values of white and brown rice treated with different preparation methods.

METHODS

Design, location, and time

The study was carried out in the Food Preparation, Nutrition, and Analytical Laboratory, Health Campus, Universiti Sains Malaysia (USM), Kelantan, Malaysia. The study was carried out from November 2022 to January 2023.

Material and tools

The main materials used for this study were WR, BR, and coconut oil. The most used WR rice (long-grain variety, 1 brand) and BR (long-grain variety, 1 brand) were purchased from a local hypermarket near to the Health Campus of Universiti Sains Malaysia which is located in the Kota Bharu district of Kelantan state, Malaysia. Two different types of rice are used: WR and BR, which are commonly consumed by Malaysians. Meanwhile, the coconut oil was purchased from a local hypermarket in Kubang Kerian, Kelantan. Chemicals and reagents used in this study were: hydrochloric acid, sulfuric acid, boric acid, red methyl, sodium hydroxide, sodium acetate, bromocrysol green, and methanol, from Merck, Germany. The selenium catalyst tablet, pancreatic enzyme, amyloglucosidase, and amylose were made by Thermofisher Scientific, United Kingdom. Acetic acid, ethanol, and potassium iodide were obtained from HmbG, Germany. Petroleum ether from JoChem Scientific & Instruments, Malaysia. The D-glucose assay kit was bought from Megazyme, Ireland. Iodine was purchased from Chemiz, Malaysia.

Procedures

Preparation of rice. Both WR and BR were then cooked with different cooking methods: (1) cooking rice without additional coconut oil and not being refrigerated (control); (2) cooking rice without additional coconut oil and being refrigerated (XCOR); (3) cooking rice with additional coconut oil and not being refrigerated (COXR); and (4) cooking rice with additional coconut oil and being refrigerated (COR) for 12 hours before performing further analyses. All cooking procedures and analyses are replicated twice.

One cup of rice (220 g) was rinsed and added to a predetermined quantity of water. For white rice, 1.5 cups of water are added, whereas for brown rice, 2 cups of water are added. In methods 3 and 4, 1 tablespoon of coconut oil was mixed with rice. Each type of rice was cooked and cooled. Rice in methods 1 and 3 was left at room temperature for 30 min, whereas rice in methods 2 and 4 was cooled for 30 min before being refrigerated (4−5°C) for 12 hours.

Analysis of proximate composition. Proximate parameters (composition of moisture, ash, protein, fat, and dietary fibre) were determined according to the AOAC methods (AOAC 2020). Meanwhile, the carbohydrate content was calculated as a hundred minus the total percentage of moisture, ash, crude protein, and fat (by-difference method). The carbohydrate content of samples was determined by calculating the percent remaining after all the other components have been measured:

$$\%\,Carbohydrates = 100 - (\%\,Moisture - \%\,Protein - \%\,Lipid - \%\,Ash)$$

Determination of amylose content. The amylose content of BR was determined by a colorimetric method with a blue amylose-iodine complex based on the method described by Jain *et al.* (2012).

Calorific value. The calorie content of the rice samples was calculated using a formula obtained from the U.S. Department of Agriculture (USDA) (2010):

$$Energy\,(kcal) = 4\,X\,Protein + 9\,X\,Fat + 4\,X\,Carbohydrate$$

In-vitro starch digestibility. eGI was determined using an assay kit GOPOD-format K-GLUC by spectrophotometry at a wavelength of 510 nm based on the method explained by Demirkesen-Bicak *et al.* (2021). One g of the sample was mixed with 5 ml of deionised water. After that, 10 ml of pepsin-guar gum solution was added, and the sample was incubated at 37°C at 175 strokes/min in a shaking water bath for 30 min. Then, 5 ml of 0.5 M sodium acetate was added, and the pH was adjusted to between 5 and 5.25. An enzyme solution of the mixture of pancreatin and amyloglucosidase (13.4 U/ml) was added, and the volume was adjusted to 50 ml using deionised water. After that, the sample was incubated for 180 min in a shaking water bath. Between the periods of incubation, 0.5 ml samples were taken at 20, 30, 60, 90, 120, and 180 min and placed in separate falcon tubes. Then the tubes were placed in a boiling water bath for 5 min to denature the enzyme. After that, the final volume of the samples was adjusted to 5 ml using deionised water and centrifuged for 5 min at 4,000X g. The eGI was calculated from the Hydrolysis Index (HI) value of each sample. The HI value was obtained through calculation by dividing the area under the hydrolysis curve with the white bread area. The eGI was then calculated using the formula below (Demirkesen-Bicak *et al.* 2021):

$$GI = 39.71 + 0.549\,X\,HI$$

Data analysis

The results were evaluated for statistical significance using the IBM Statistical Package for Social Sciences (SPSS) Statistics Data Editor Version 26. The results were conveyed as means±standard deviation. A p-value of less than 0.05 was deemed significant. The differences between the means were analysed for significance using a one-way analysis of variance (ANOVA) test by employing the post-hoc Tukey test.

RESULTS AND DISCUSSION

Moisture content. Moisture content in food can be defined as water left within the food, indicating an index of its nutrient content (Kim & Lee 2013). The moisture contents of the WR were 62.07, 62.74, 67.00, and 68.46%, respectively, whereas the moisture contents of the BR were 61.57, 63.54, 66.91, and 69.28% respectively (Table 1). These results showed that the WR had a relatively higher moisture content than the BR in preparation methods 1 and 3. Since the BR has a high-fibre bran, more water is needed to cook BR, and it is demonstrated that the bran layer inhibits moisture absorption (Chapagai *et al.* 2020). Plus, it may be due to the drying effects of the exothermic reaction of the dehusking procedure (Kim & Lee 2013). On the other hand, in preparation methods 2 and 4, BR shows that it has a relatively higher moisture content than WR. A previous study stated that the moisture content had little effect on the amylose recrystallisation (Ding *et al.* 2019). Thus, BR, which already contains more amylose concentration, has a higher moisture content to undergo amylose recrystallization during the retrogradation process.

For further analyses of ash, protein, and fat contents, each rice sample was ground first before being analysed; the results showed that there are

Table 1. The proximate composition of WR and BR prepared with different methods

Nutritional composition	Concentration (%)			
	Control	2 (XCOR)	3 (COXR)	4 (COR)
Moisture				
WR	62.07±0.11	62.74±0.13	67.00±0.10	68.46±0.08
BR	61.57±0.12	63.54±0.12	66.00±0.09	69.28±0.10
Ash				
WR	0.24±0.01^{b}	0.28±0.02^{b}	0.29±0.09^{b}	0.24±0.04^{b}
BR	1.17±0.02	1.13±0.01	1.17±0.07	1.08±0.06
Fat				
WR	0.50±0.02^{b}	0.20±0.02^{b}	4.10±0.09^{b}	3.80±0.04^{b}
BR	2.47±0.03	1.91±0.03	5.78±0.08	5.37±0.06
Protein				
WR	9.46±0.11	9.45±0.09	8.86±0.11	8.58±0.11
BR	8.91±1.12^{b}	8.85±0.08^{a}	8.63±0.10a	8.16±0.10^{a}
Carbohydrate				
WR	27.73±0.18	27.33±0.11	19.73±0.14	18.88±0.12
BR	25.88±0.13^{b}	24.58±0.13^{a}	17.52±0.08^{b}	16.10±0.11^{b}
TDF				
WR	2.90±0.01^{b}	2.93±0.02^{b}	4.00±0.07^{b}	6.20±0.05^{b}
BR	4.90±0.02	4.82±0.03	5.60±0.05	8.01±0.09
Calorie (kcal/100 g)				
WR	344.72±1.10^{b}	285.61±1.15^{a}	248.00±1.10^{a}	223.93±0.09^{a}
BR	350.50±1.12^{a}	267.85±1.13^{b}	195.67±0.09^{b}	169.91±0.15^{b}

$^{a-b:}$ Mean±SD with different superscript letters within the same row indicate a significant difference ($p<0.05$)

WR: White Rice; BR: Brown Rice; TDF: Total Dietary Fibre

1 (Control): Rice cooked without additional of coconut oil and not being refrigerated

2 (XCOR): Rice cooked without additional of coconut oil and being refrigerated

3 (COXR): Rice cooked with additional of coconut oil and not being refrigerated

4 (COR): Rice cooked with additional of coconut oil and being refrigerated

different trends in analysing ash, protein, and fat content in the cooked rice.

Ash content. The ash content refers to the essential mineral residue remaining from burning organic matter at high temperatures. The higher the ash content of the food, the more mineral elements it contains. The ash content in the BR varied between 1.080–1.173% and the WR was between 0.239–0.290%. The result shows that the ash content was higher in the BR than in the WR. WR contains less ash content because the milling processes it undergoes, such as bran removal, have affected its mineral content (Helmyati *et al.* 2020). On the other hand, the result in Table 1 also shows that there is a decreasing pattern of ash content for both types of rice, WR and BR, that undergo refrigeration. According to the previous study by Helmyati *et al.* (2020), it demonstrated that a decrease in ash content in food may be due to the susceptibility of ash to storage conditions. It also might be caused by the physiological activities of the mineral, possibly due to the respiration process that subsequently decreases the mineral content in the rice samples (Nur Shafinaz *et al.* 2022). However, some minerals can be volatilized or reduced when heated at high temperatures during the drying process, particularly vitamins B and C (Chapagai *et al.* 2020). This study shows that the storage temperatures indirectly promote the physiological process and affect the ash content of rice samples.

Protein content. The protein content for the WR ranged from 8.58% to 9.46%, while the BR evaluated ranged from 8.16% to 8.91%. According to the Malaysian Food Composition Database (MyFCD 1997), domestic white rice contains a lower protein content with a value of 7.1 g/100 g while BR of Jasmine's production contains 8 g/100 g. Generally, WR has a higher protein concentration than BR in all preparation methods (Table 1). A previous study demonstrated that more pigmented rice tends to have reduced protein digestibility (Yuliana & Akhbar 2020). This supports our study's result which shows BR has a lower protein concentration than WR. Protein availability depends on several factors such as heat, the presence of moisture, reducing substances, and the duration of heating (Helmyati *et al.* 2021). The amino group of protein is attached to the carboxyl group through peptide bonds due to the loss of water molecules during the dehydration process (Schmeing 2013). However, this peptide bond can also be broken down to release the amino group through the hydrolysis process. This may happen during the cooking process of rice samples (Chapagai *et al.* 2020). The prolonged cooking may cause the physical structure of the protein to denature and no longer function (Sanfelice & Temussi 2016). Proteins are highly sensitive to temperature, both cold and hot, which may alter the structure of the protein, denature it, and make it non-functional (Sanfelice & Temussi 2016). Protein denaturation is not strong enough to disrupt the peptide bonds in the primary structure of the protein, but it can affect the secondary and tertiary structures of the protein (Amagliani *et al.* 2017). As the protein in rice samples is the secondary structure of the protein, the peptide bonds may easily be broken down.

It also shows that there is a decreasing trend in protein concentration with the preparation method. Protein unfolding may also be due to cold denaturation when proteins at room temperature are cooled to lower values (Sanfelice & Temussi 2016). Nonpolar groups of proteins tend to avoid contact with water to engage in hydrophobic interactions because, at low temperatures, the entropic advantage of the water molecule in folding non-polar groups of the protein inward is lessened (Taborsky 1979). This produces an endothermic reaction with low kinetic energy that may alter the protein concentration of refrigerated rice samples.

Fat content. All rice is made up primarily of carbohydrates, with small amounts of protein and practically low-fat content (Chapagai *et al.* 2020). Brown rice contains a good source of linoleic acid and essential fatty acids and does not contain cholesterol (Chapagai *et al.* 2020). Table 1 shows that the fat concentration of WR ranged between 0.20% and 4.12%, whereas that of BR ranged between 1.91% and 5.78%. Overall, low fat content was recorded in the WR variety (0.20%), significantly lower than the BR variety. As BR is categorised as a whole grain, the germ part contains fatty acids, making it contain more fat from the essential oil. It also shows that rice that was refrigerated had a lower fat concentration than rice that was not refrigerated. This result may be due to the formation of a starch-lipid complex in a starch-water system during the retrogradation process (Arik Kibar *et al.* 2013). A previous study stated that the more the amylose-fatty acid complex forms, the more unsaturated fatty acids are introduced to the amylose of starch (Arik Kibar *et al.* 2013). However, with the addition of the coconut oil during cooking (samples 3 and 4), the fat percentage in the rice samples increased by approximately 3 times that of the control sample. The crude fat content of coconut oil was higher in relation to the fat content of rice. However, even though coconut oil is highly saturated, its predominance of medium-chain fatty acids causes it to have a different metabolic behaviour (Santos *et al.* 2020).

Carbohydrate content. Carbohydrate content in food, including organic acids, polyols, and dietary fibre, contributes to the energy content, and it is important to provide the energy required by humans (Santos & Timoteo 2016). For carbohydrate content, the values shown in Table 1 were in the range of 18.88% to 27.73% for WR and in the range of 16.10% to 25.88% for BR. According to the MyFCD (1997), white rice contains a lower carbohydrate content with a value of 79 g/100 g, while brown rice from Jasmine's products contains 74 g/100 g. This result is consistent with the results obtained from our study, showing that BR has a lower carbohydrate concentration compared to WR. During the cooking process, the excess sugars from the starch may be separated from the rice and then converted into gelatinous liquid in the upper inner lid. This will produce rice with less carbohydrate content (Lauben 2022). Table 1 also shows that the carbohydrate content is decreasing with the refrigeration of rice samples for both rice types. Many factors influence carbohydrate digestion, including the type of starch and the type of preparation. During refrigeration, the retrogradation process occurs, and amylose is involved in this process. Amylose is a starch that accounts for approximately 20% of the dietary carbohydrate (Dworken *et al.* 2020). The high concentration of carbohydrates resulted in an increase in amylase activity to break down carbohydrates into simple sugar. This means that, in this study, carbohydrate, which is supposedly higher in amylopectin than amylose concentration, was rearranged to have more amylose concentration than amylopectin, thus having a lower carbohydrate content than usual (Ding *et al.* 2019). Low-carb foods tend to break down slowly during digestion. Thus, it is considered a low-GI food (Kaur *et al.* 2016). The addition of coconut oil during the cooking process showed that the use of coconut oil does not contribute to the carbohydrate concentration in the rice because it does not contain carbohydrates.

TDF content. There were significant differences ($p<0.05$) in the TDF content of WR and BR cooked with different methods. The presence of TDF in the diet is essential to increase the bulk of faeces because it has a laxative effect on the gut (Kaur *et al.* 2016). Generally, the BR samples have more TDF composition as compared to WR samples. The composition of TDF for WR treated with COXR and COR (4.0% and 6.2%) was significantly ($p<0.05$) lower than for BR treated with COXR and COR (5.6% and 8.0%), respectively. BR is known to be a good source of dietary fibre, and it was the highest in this study. The finding indicates that the BR has a higher TDF content than the WR. It is because BR is a whole-grain food containing bran and germ (Santos & Timoteo 2016). These parts specifically provide more fibre, vitamins, and minerals for those who consume BR. This also putatively elicits differential effects on satiety that are very advantageous in the regulation of energy intake as well as body weight (Sanfelice & Temussi 2016). In a comparison of methods 3 (COXR) and 4 (COR), there is an increasing pattern in the TDF concentration with the refrigeration method. During the refrigeration process, starch retrogradation occurs, resulting in an increase in TDF content. It happens when the available and digestible starches are converted into resistant starches, which are also considered dietary fibres because they are not broken down into glucose (Kaur *et al.* 2016). Refrigerated rice contains more Resistant Starch (RS) than unrefrigerated rice (Sanfelice & Temussi 2016). The use of coconut oil in this study also showed that coconut oil increases the TDF content of rice. It is because more amylose-lipid complexes are formed during the cooking process, producing RS type 5 (Birt *et al.* 2013).

In our present study, a positive correlation was observed in Table 2, wherein rice samples had a higher amylose content corresponding with the preparation methods. BR has a higher amylose concentration approximately 10 times (ranging between 91.39% and 113.3%) than WR (ranging between 3.94% and 9.43%). The higher the amylose content in the rice sample, the higher the starch retrogradation in the rice sample,

Table 2. The amylose concentration in WR and BR with different preparation methods

	Amylose concentration (%)			
	Control	2 (XCOR)	3 (COXR)	4 (COR)
Moisture				
WR	3.94±0.08	4.21±0.12	8.59±0.03	9.43±0.09
BR	91.39± 3.87	97.50±1.87	100.73±1.20	113.53±2.95

WR: White Rice; BR: Brown Rice

1 (Control): Rice cooked without additional of coconut oil and not being refrigerated

2 (XCOR): Rice cooked without additional of coconut oil and being refrigerated

3 (COXR): Rice cooked with additional of coconut oil and not being refrigerated

4 (COR): Rice cooked with additional of coconut oil and being refrigerated

which will subsequently increase the RS content (Helmyati *et al.* 2020). The increase in RS content in food lowers the eGI and calories of the food.

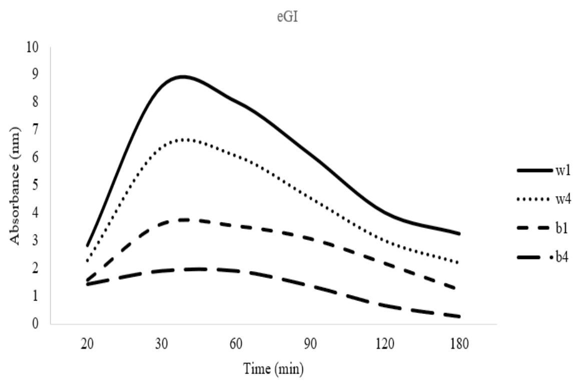
Energy value. Energy value measures the available amount of energy obtained from food via cellular respiration. In this study, Table 1 shows that BR with preparation method 1 (350.50 kcal) has the highest energy per 100 g among all samples analysed compared to the WR (344.71 kcal). According to the MyFCD (2003), medium-grain WR contains approximately 343 kcal, whereas the BR of Jasmine SunBrown's production contains 353 kcal. Even though BR is more nutritious, it contains more calories than WR. It is because the calorie content of food is determined by the fat, protein, and carbohydrate in the food (Rondanelli *et al.* 2019). As BR is wholegrain food, it has the germ and bran intact. Rice germ contains high levels of protein, fatty acids, and fibre, which are likely to contribute to the total calorie content of the food (Rondanelli *et al.* 2019). For this reason, the WR sample has fatty acid-rich germ removed during the milling process and contains fewer calories than the BR.

Above that, rice that has been refrigerated and rice with additional coconut oil have fewer calories because the DS is being converted into RS during starch retrogradation (Wang *et al.* 2015). RS contains fewer calories (2 kcal/g) than DS (4 kcal) (Maya *et al.* 2020). This can lower the calories consumed with the same food volume in each food intake. However, the rice sample of BR with preparation method 4 also has the lowest total energy per 100 g (169.91 kcal) than the WR (223.93 kcal).

Methods of food processing such as refrigeration affect the RS content of the food. According to the American Chemical Society (ACS 2015), the cooling process is essential because amylose from starch leaves the granules during gelatinisation to form hydrogen bonds between the amylose molecules outside the granule, so that it becomes resistant to the digestive enzyme (Wang *et al.* 2015). That is how RS is formed. WR, which is primarily known as a high-GI food, swaps to rice with a lower GI because of the starch retrogradation involving amylose reorganisation, which has changed the starch into RS or fibre (Ding *et al.* 2019).

The addition of coconut oil during the cooking process makes the oil enter the starch granules, and adding a layer to the starch makes it resistant to the digestive enzyme (Birt *et al.* 2013). The formation of amylose-lipid complexes restricts the swelling of the starch granules and the hydrolysis of the enzyme (Birt *et al.* 2013). Thus, in this situation, the starch granules have been converted into thermally stable RS type 5, which is formed through cross-linking of amylose-lipid.

The eGI. The GI of food is determined by its ability to raise blood glucose (Wang *et al.* 2015). The graph in Figure 1 shows that the eGI of BR is lower than the WR. This indicates the lower postprandial phase of hunger. It may be due to the lesser and slower breakdown of the BR than the WR. This difference appears to be influenced by the bran layer of the BR, which inhibits gastric acid absorption and slows gastric emptying, which manifests in the consequential state of satiety (Kaur *et al.* 2016). Besides, the higher the Resistant Starch (RS) contained in the rice sample, the lower the eGI it has. The higher RS content decreased its available carbohydrate content because RS is classified as an unavailable carbohydrate as it cannot be digested and



w1: White rice cooked without addition of coconut oil and not being refrigerated (control)
w4: White rice cooked without addition of coconut oil and being refrigerated
b1: Brown rice cooked with addition of coconut oil and not being refrigerated
b4: Brown rice cooked with addition of coconut oil and being refrigerated

Figure 1. The eGI of total starch in WR and BR cooked with different preparation methods

absorbed in the SI (Wang *et al.* 2015). Unlike the DS, RS is not broken down in the SI, where the carbohydrates are normally metabolised into glucose to be absorbed into the bloodstream. Thus, reducing the higher peak of blood glucose after a meal.

Since data is normally distributed, the Pearson correlation test was used to measure the strength and direction of the association between amylose concentration and eGI. The result showed a significant reduction in the eGI with the addition of amylose. Therefore, there is a significant relationship between amylose concentration and eGI. The result from simple linear regression analysis has shown that there is a significant, very strong, and negative linear relationship between amylose concentration and eGI (Table 3), tested using Pearson's correlation test ($p<0.001$, $r=-0.935$).

Since data is not normally distributed, Spearman's rank correlation test was used to measure the strength and direction of the association between amylose concentration and calories (Table 3). The result showed a significant reduction in calories with the addition of amylose concentration. Thus, there is a significant relationship between amylose concentration and

Table 3. The relationship between eGI and calories with different amylose concentrations

	eGI			Calories		
	n	r	*p*	n	r	*p*
Amylose concentration	4	-0.935	<0.001$^{\alpha}$	4	-0.634	<0.001$^{\alpha}$

α: Pearson Correlation; eGI: estimated Glycaemic Index

calories. The result from simple linear regression analysis has shown that there is a significant, strong, and negative linear relationship between amylose concentration and calories, tested using Spearman's rank correlation test ($p<0.001$, $r=-0.634$).

CONCLUSION

The methods of cooking rice with the addition of coconut oil and/or refrigerated rice were some of the alternative preparation techniques to lower both the calorific value and glycaemic index for the preparation of healthier rice meals. Additionally, the favourable effects on lowering calories and glycaemic index may be due to the increase in resistant starch (RS3 and RS5) contained in the cooked rice. Consuming refrigerated rice that has been cooked with coconut oil can be used as an alternative preparation technique to lower both the calorific value and the glycaemic index for the preparation of healthier rice meals for health-conscious individuals. In conclusion, the rice cooked with additional of coconut oil and being refrigerated (COR) method can be recommended to produce the lowest eGI for both WR and BR. Alternatively, the consumers may apply this COR method to cook BR to produce healthy rice not only with low in eGI and caloric value but also high in DF.

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DECLARATION OF INTERESTS

The authors have no conflicts of interest.

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The Therapeutic Effects of West Indian Elm (*Guazuma ulmifolia*) Leaf Extract on Coronary Artery Atherosclerosis in Hypercholesterolemic Wistar Rats

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ABSTRACT

This study aimed to determine the therapeutic effect of West Indian elm leaf extract on coronary artery atherosclerosis in hypercholesterolemia Wistar rats. Cholesterol, a key factor in the pathophysiology of coronary heart disease, is present in fat-containing diets. Orlistat is one of the medications that can reduce blood cholesterol levels, but since it has side effects, herbs are more preferable to it. One such herbal plant, West Indian elm (*Guazuma ulmifolia*), contains tannins, saponins, alkaloids, and flavonoids, secondary metabolites that carry the properties necessary to reduce blood cholesterol levels, in its leaves. In this experimental study, which focused on histopathological parameters to see the scale of progression of coronary artery atherosclerosis, 30 Wistar rats were induced with a high-fat diet and divided into 6 sample groups, consisting of three control groups (normal, negative, and positive) and three treatment groups (which received a West Indian elm leaf extract at 0.2, 0.4, and 0.8 g/kgBW). Significant results were obtained from both Kruskal-Wallis and Mann-Whitney tests ($p<0.05$) between the negative control group and the positive control group, between the negative control group and treatment group 2 (0.4 g/kgBW), and between the negative control group and treatment group 3 (0.8 g/kgBW). In conclusion, this study proved that West Indian elm (*Guazuma ulmifolia*) leaf extract reduces the development of coronary atherosclerosis and shares a similarity in therapeutic effect with orlistat.

Keywords: atherosclerosis, histopathology, orlistat, west indian elm leaf

INTRODUCTION

People who frequently consume fast foods, eat less fruits and vegetables, engage in less physical activity, drink alcohol and too much soda, and smoke cigarettes are pursuing an unhealthy lifestyle. Such a way of living affects their likelihood of developing heart disease, particularly Coronary Heart Disease (CHD), which is caused by the existence of obstructive atherosclerotic plaques that reduce the blood flow to the heart's myocardium (Severino *et al.* 2020). According to the findings of a 2017 research study, cardiovascular diseases were responsible for 17.8 million global deaths, and 35.6 million people survived with impairments (Roth *et al.* 2020). Coronary heart disease is seen as posing a serious danger to the 21st-century sustainable development (Khan *et al.* 2020).

The proportion of the national population consuming fatty foods once a day was reported to be fairly high at 40.7% (Zulkarnain 2018). Foods high in saturated fatty acids or trans-fatty acids (i.e., red meat, full-fat dairy products, butter, and coconut oil) cause an increase in lipid levels in the blood (Schoeneck & Iggman 2021). In fact, fatty foods contain cholesterol, which is an important factor in the pathogenesis of coronary heart disease (Kandaswamy & Zuo 2018). High intake of saturated fat can increase Low-Density Lipoprotein (LDL) (Nurdin *et al.* 2016) in the bloodstream and cause atherosclerotic plaques to form within the walls of the arteries (Jesch & Carr 2017).

Numerous clinical trials have demonstrated that lowering LDL-C (cholesterol-rich lipoprotein) levels can lower the risk of cardiovascular events (Lechner *et al.* 2020). One medication, orlistat, deactivates lipase by covalently attaching to the serine residue in the active site. Triglyceride breakdown is prevented by lipase inactivation, and as a result, free fatty

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acids are not absorbed (Bansal & Khalili 2022). However, because medications, especially orlistat, can have side effects like osteoporosis, stature problem, and acute kidney failure, it is crucial to learn more about natural food ingredients that can inhibit cholesterol absorption so that food ingredients and food supplements can be developed to treat people who wish to manage plasma cholesterol levels through non-pharmacological therapy (Jesch & Carr 2017).

West Indian elm (*Guazuma ulmifolia*), belonging to the family Sterculiaceae, is a small genus of trees native to tropical America. Indonesian people have used the plant as traditional medicine, believing that the leaves can be used for slimming, the seeds can be used for treating diarrhea, constipation, and flatulence, the bark can be used as a diaphoretic, and the fruits or leaves can be used as treatment for diarrhea, cough, and abdominal pain and as tonic and astringent (Lumbantobing *et al.* 2019). According to research findings, West Indian elm has been utilized as hepatoprotective, anti-cancer, anti-obesity, antioxidant, and antibacterial medication. In animals with hyperlipidemia, West Indian elm can also lower blood cholesterol levels and the occurrence of atherosclerosis (Prahastuti *et al.* 2020) because the secondary metabolites found in the leaves and fruits, such as alkaloids, tannins, saponins, flavanoids, terpenoids, and steroids, are significant substances used in medicines (Kumar & Gurunani 2019). There has been a great deal of additional research on West Indian elm leaves in Indonesia. The majority of the research addresses West Indian elm leaves as an obesity therapy. However, not much of the research is associated with atherosclerosis, especially in the coronary arteries. In order to assess the therapeutic impact of West Indian elm leaf extract, this study concentrated on the histological characteristics of the coronary arteries of Wistar rats (*Rattus norvegicus*) living on a high-fat diet.

METHODS

Design, location, and time

The research is of a true experimental type with a randomized posttest-only control group design (the sample was randomly divided into groups, and the research data were taken after treatment). The research was conducted in the laboratory using experimental animals (in vivo). The purpose of this study is to analyze the existence of a causal relationship between the treatment (the administration of West Indian elm leaf extract) and the output (the histopathological images of the coronary arteries of the experimental animals). The preparation of the West Indian elm (*Guazuma ulmifolia*) leaf extract and the phytochemical analysis were carried out at BALITTRO, Bogor, and the experimental animal treatment was administered at the Laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, Padjadjaran University, Bandung. The study was carried out between April 2022 and December 2022. This research received an ethical clearance from the UPN "Veteran" Jakarta Ethics Committee (No: 393/IX/2022/KEPK).

Materials and tools

West Indian elm leaf extract. The extract was prepared through maceration with 96% ethanol and evaporation. In addition to 178.4 g of crude simplicia, 2,000 g of dried West Indian elm was used as raw material. With these amounts of raw material and simplicia, the yield was calculated using the following formula.

$$Yield = \frac{Extract\ weight}{Dry\ leaves\ weight} X100\%$$

Phytochemical analysis. After simplicia was obtained from maceration, a phytochemical analysis was carried out to see the secondary metabolite contents of the extract. It was conducted following the test method explained in 1995 MMI Volume VI guidelines.

Orlistat. In this study, orlistat was administered to experimental rats at 2.16 mg three times a day. This dose was determined in proportion to the dose given to humans (120 mg, three times a day) (Qi 2018).

High-fat diet preparation. According to the Faculty of Medicine of Padjadjaran University (FK UNPAD) (2016), the high-fat diet given to the experimental rats was made of 8 kg of standard pellets, 15 duck egg yolks, 2.5 kg of flour, 0.75 l of coconut oil, 1 kg of goat fat, 3 g of cholesterol medication, 15 quail eggs, and enough hot water.

Procedures

Determination of sample size and inclusion criteria. The sample size used was determined using Federer's formula (Widiyatno

& Muniroh 2018). Thirty male Wistar rats (*Rattus norvegicus*) weighing 150–250 g and aged around 8 weeks (Thadeus *et al.* 2019) were utilized in this study. At 8–10 weeks, Wistar rats (*Rattus norvegicus*) reaches adulthood as they are ready for reproduction (Sengupta 2013).

Acclimatization of experimental animals. Rats were kept in 150 cm^2 rectangular plastic cages to which sand was spread, with a covering of ram wire, to adapt for seven days. Every five rats were kept in one cage with a light intensity in the range from 1 to 25 lux. The lighting was set to be on for 12 hours and off for another 12 hours. The cage environment was kept from being damp, with its temperature maintained at around 25^{o}C. The rats were given standard feed and water ad libitum. During the acclimatization period, the rats were given 551 pellets and distilled water. After the acclimatization period was over, the rats were then treated according to their group.

Treatment groups. The sample was divided into three control groups (normal, negative, and positive) and three treatment groups (given West Indian elm leaf extract at different doses). Each group contained five rats. Group K0 (normal control) was given normal feed and water for 56 days. Group K1 (negative control) was given a high-fat diet for 56 days. Group K2 (positive control) was given a high-fat diet for 56 days and 2.1 mg of orlistat medication three times a day from day 29 to day 56. Groups K3, K4, and K5 (treatments 1, 2, and 3) were given a high-fat diet for 56 days and West Indian elm leaf extract at 0.2, 0.4, and 0.8 g/kgBW once a day, respectively, starting from day 29 to day 56.

Termination and surgery. After the rats underwent their respective experimental treatments, they were given 0.3 ml of ketamine by intramuscular injection to put them to sleep. Ketamine was selected because it is simpler to administer, acts quickly, and has a potent analgesic impact (Yulianti & Astari 2020). The rats were terminated and dissected, and, after that, the coronary artery of each rat (in the rat's heart) was extracted.

Preparation and staining of slides. The paraffin technique was used to prepare histological slides in several steps, namely, dehydration, fixation, clearing, infiltration, cutting, attachment, and deparaffinization (Harijati *et al.* 2017). Furthermore, Hematoxylin and Eosin (HE) staining was used to stain the samples (Cardiff *et al.* 2014). A 400x magnifying microscope was used to read the histopathological images.

Determination of coronary artery atherosclerotic lesion indicators. Atherosclerotic lesion indicators were used to determine the degree of coronary artery damage. Score 0 indicates that the histological image was in the normal condition. Score 1 indicates that there was an atherosclerotic plaque less than half the thickness of the tunica media with some form of endothelial dysfunction. Score 2 indicates that there were atherosclerotic plaques at least half the thickness of the tunica media with an accumulation of intracellular lipids, macrophages, and smooth muscle cells. Score 3 indicates that there were atherosclerotic plaques as thick as the tunica media with numerous macrophages, smooth muscle cells, and connective tissue. Score 4 indicates that atherosclerotic plaques were found to be thicker than the tunica media with a large intracellular lipid core and inflammatory cell infiltration (Kabiri *et al.* 2011).

Data analysis

The IBM® SPSS® Statistics computer program, version 24.0, was used to process all of the collected data. This study's data analysis applied the Kruskal-Wallis test to determine whether there was an average difference between at least two group pairs. The next test was the Mann-Whitney post-hoc test, aimed to get the average difference between each pair of groups. The confidence interval of both tests was 95%.

RESULTS AND DISCUSSION

West Indian elm leaf extract

The yield of West Indian elm (*Guazuma ulmifolia*) leaf extract in this study was 8.92%. Yield is essential in making extracts because the yield value is related to the number of bioactive compounds in the extract (Whika *et al.* 2017). The higher yield value, the greater the resulting extract. This means that more bioactive compounds are contained in plant leaves (Nahor *et al.* 2020). The results of the phytochemical analysis of West Indian elm (*Guazuma ulmifolia*) leaf extract in this study are presented in Table 1.

Table 1 shows that the West Indian elm (*Guazuma ulmifolia*) leaf contains secondary metabolites such as saponins, tannins, alkaloids, phenolics, triterpenoids, steroids, glycosides,

Table 1. Phytochemical analysis test

Phytochemical analysis	Results
Alkaloid	+
Saponin	+
Tannin	+
Phenolic	+
Flavonoid	+
Triterpenoid	+
Steroid	+
Glycoside	+

(+): Positive; (-): Negative

and flavonoids. Since this study used qualitative phytochemical analysis, the results were either positive or negative to indicate the presence of secondary metabolites. Secondary metabolism describes metabolic pathways and the tiny molecules they produce that are unnecessary for the organism's growth and reproduction. In plants, secondary metabolic pathways produce a diversity of compounds called Plant Secondary Metabolites (PSMs). Many PSMs have positive, beneficial effects on human health and agriculture production, contributing significantly to the economy (Pang *et al.* 2021).

Histopathological image results

Histopathological observations of the coronary arteries of rats were interpreted with indicators of atherosclerotic lesions in the coronary arteries. The atherosclerotic indicators referred to are presented in Table 2.

Based on Table 2, there was no progression of coronary artery atherosclerosis in the normal control group because all the rats in the group had a progression score of 0. The normal control group was fed with sodium carboxymethyl cellulose at all times. This group represented a disease-free state against which the intervention groups were to be compared.

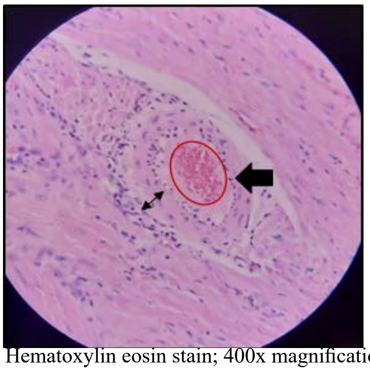
The progression results show no changes in the architecture of the coronary arteries of the rats because there were no risk factors to induce it (Figure 1). Hyperlipidemia and hyperglycemia are significant risk factors for the pathogenesis of atherosclerosis because they increase Reactive Oxygen Species (ROS) levels, thereby damaging the vascular endothelium (Rafieian-Kopaei *et al.* 2014).

The negative control group was a group of rats that were given a high-fat diet with the addition of neither orlistat nor West Indian elm leaf extract. Based on Table 2, the highest atherosclerosis progression was scored by the negative control group. This is because the high-fat feed given contained many lipid-derived macromolecules, such as triacylglycerols, cholesterol, and fatty acids, which would then be absorbed by the small intestine and enter the blood circulation. In this study, the risk factor for atherosclerosis in the coronary arteries of rats was hypercholesterolemia. High levels of cholesterol, especially LDL, followed by chronic inflammation of the endothelium, will cause endothelial dysfunction. The injured endothelium can be penetrated by circulating LDL in the blood vessels, which will enter the space in the

Table 2. Results of atherosclerotic lesion assessment indicators in coronary arteries

Nomenclature	Groups	Score				
		0	1	2	3	4
K0	Normal control	5	-	-	-	-
K1	Negative control	-	-	5		-
K2	Positive control	4	-	1	-	-
K3	Treatment 1	1	-	4	-	-
K4	Treatment 2	2	-	3	-	-
K5	Treatment 3	3	1	1	-	-

K0: Normal feed and water
K1: High-fat diet
K2: High-fat diet and 2.1 mg of orlistat
K3: High-fat diet and West Indian elm leaf extract 0.2 g/kgBW
K4: High-fat diet and West Indian elm leaf extract 0.4 g/kgBW
K5: High-fat diet and West Indian elm leaf extract 0.8 g/kgBW



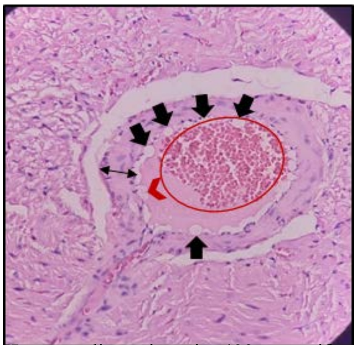
Hematoxylin eosin stain; 400x magnification

○ : Lumen filled with erythrocytes
⬅ : Tunica intima endothelium
↔ : Tunica media

Figure 1. Picture of atherosclerosis progressive score 0

subintima. This will give rise to atheroma plaques (Aziz & Yadav 2016).

Lipid accumulation in the subintima space is clearly visible in the histopathological image of the coronary arteries of the negative control group rats (Figure 2). Progressive lesions in the negative control group did not reach score 3 (marked by atherosclerotic plaques as thick as the tunica media and the presence of inflammatory cells and smooth muscle cell proliferation) or score 4 (marked by atherosclerotic plaques thicker than the tunica media and lots of inflammatory cell infiltration). This was because the level of fat in the diet consumed by the experimental rats during the 56 days' duration was not significant enough to cause atherosclerotic lesions.



Hematoxylin eosin stain; 400x magnification

❮ : Endothelial dysfunction
⬅ : Vacuoles contain intracellular lipids
↔ : Tunica media
○ : Lumen filled with erythrocytes

Figure 2. Picture of atherosclerosis progressive score 2

The positive control group, which was given a high-fat diet and an orlistat therapy at a dose of 2.16 mg three times a day, had low atherosclerosis progression, almost equivalent to the normal control group. Based on Table 2, only 1 rat in the group demonstrated atherosclerosis progression, while 4 others were normal. The positive control group proved that orlistat can reduce the progression of atherosclerosis. This is because orlistat covalently binds to the serine residue of the lipase active site and deactivates it. Lipase inactivation prevents triglyceride hydrolysis, and thus free fatty acids are not absorbed (Bansal & Khalili 2022). By decreasing the absorption of fat in the intestine, total cholesterol levels in the blood will also decrease, so that the risk factors for atherosclerosis in the coronary arteries can be inhibited. One rat that experienced atherosclerosis progression, K2-4, which scored 2 in the analysis, already had a greater body weight than the other rats from the beginning of the study. At the end of the study, it weighed 381 g, making it the rat with the greatest body weight in this study. Based on what happened to these rats, it can be concluded that the risk factors for atherosclerosis can also come from sources that cannot be modified, namely genetics, age, and sex (Johansson *et al.* 2021).

The results of progressive atherosclerotic lesion analysis of treatment groups 1, 2, and 3 show that the administration of West Indian elm leaf extract at doses of 0.2, 0.4, and 0.8 g/kgBW, respectively, had a therapeutic effect. Based on Table 2, treatment group 3 had the following results: one rat had a lesion progression score of 2, one had a lesion progression score of 1, and three had a lesion progression score of 0. These results were better than those of treatment groups 1 and 2. Although treatments 1 and 2 were considered ineffective in inhibiting the progression of atherosclerotic lesions, they had lower scores than the score of the negative control group. This means that at doses of 0.2 and 0.4 g/kgBW, West Indian elm leaf extract already showed a therapeutic effect.

The West Indian elm leaf extract contains secondary metabolites such as tannins, alkaloids, and saponins (Kumar & Gurunani 2019). These compounds can bind to cholesterol and fat molecules in food, reducing the amount of fat and cholesterol absorbed in the intestine. This will cause a decrease in cholesterol in the blood (Rozqie *et al.* 2012). In addition, the West Indian elm leaf extract also contains flavonoids, which act as antioxidants so as to prevent endothelial damage due to oxidative stress and prevent LDL oxidation. Flavonoids also act as anti-inflammatory, cholesterol-lowering, antihypertensive, and antiplatelet agents. They can also inhibit the proliferation of smooth muscle cells that occur in the pathogenesis of atherosclerosis (Ciumărnean *et al.* 2020). The secondary metabolites contained in the West Indian elm (*Guazuma ulmifolia*) leaf extract have different mechanisms of action in inhibiting the progression of atherosclerosis, so they are effective for use as therapy.

Atherosclerotic lesion assessment indicator data were analyzed using the Kruskal-Wallis test, yielding asymp. sig. results with $p<0.05$. This analysis was followed by the Mann-Whitney test to see the average difference of each experimental group (Table 3).

A comparison of the Mann-Whitney post-hoc test results is presented in Table 3. The negative control group, which was the group with coronary artery atherosclerosis, was used as a benchmark to for the therapy groups. There were significant average differences between the negative control group and the positive control group, between the negative control group and treatment group 2, and between the negative control group and treatment group 3, statistically proving that orlistat and West Indian elm (*Guazuma ulmifolia*) leaf doses of 0.4 and 0.8 g/kgBW were effective in inhibiting the progression of atherosclerosis. In addition, the average difference was not significant between the negative control group and treatment group 1. This means that the administration of the West Indian elm (*Guazuma ulmifolia*) leaf extract at a dose of 0.2 g/kgBW was effective as therapy. Based on descriptive data and analytic tests, the most effective dose to provide a therapeutic effect on the progression of coronary artery atherosclerosis, and thus prevent and treat coronary heart disease, was 0.8 g/kgBW.

Based on Table 3, there were no significant average differences ($p>0.05$) between the normal control group and treatment group 3 ($p=0.136$) and between the positive control group and treatment group 3 ($p=0.606$). This statistically proves that the administration of West Indian elm (*Guazuma ulmifolia*) leaf extract at a dose of 0.8 g/kgBW had an effect equivalent to that of orlistat in inhibiting the progression of atherosclerosis. In addition, the West Indian elm (*Guazuma ulmifolia*) leaf extract dose of 0.8 g/kgBW did provide a therapeutic effect, so that the histopathological appearance of the coronary arteries in rats induced with a high-fat diet did not differ significantly from the histopathological appearance of the coronary arteries in normal rats. In other words, the West Indian elm (*Guazuma ulmifolia*) leaf extract dose of 0.8 g/kgBW was the most effective in providing a therapeutic effect on the progression of coronary artery atherosclerosis because the results were not significantly different from the results of rats given orlistat at 2.16 mg three times a day or the results of healthy rats. From

Table 3. Mann-Whitney test of atherosclerotic lesion assessment indicators in coronary arteries

Rats (n=5)	Mean±SD	Normal control	Negative control	Positive control	Treatment 1	Treatment 2	Treatment 3
Normal control	0±0.000	-	p=0.003	p=0.317	p=0.014	p=0.050	p=0.136
Negative control	2±0.000	-	-	p=0.014	p=0.317	p=0.134	p=0.017
Positive control	0.4±0.894	-	-	-	p=0.072	p=0.221	p=0.606
Treatment 1	1.6±0.894	-	-	-	-	p=0.513	p=0.106
Treatment 2	1.2±1.095	-	-	-	-	-	p=0.356
Treatment 3	0.6±0.894	-	-	-	-	-	-

K0: Normal feed and water

K1: High-fat diet

K2: High-fat diet and 2.1 mg of orlistat

K3: High-fat diet and West Indian elm leaf extract 0.2 g/kgBW

K4: High-fat diet and West Indian elm leaf extract 0.4 g/kgBW

K5: High-fat diet and West Indian elm leaf extract 0.8 g/kgBW this study, at a dose of 0.8 g/kgBW, West Indian elm leaf extract could slow the development of coronary artery atherosclerosis in Wistar rats (*Rattus norvegicus*) fed with a high-fat diet.

CONCLUSION

Secondary metabolites like saponins, tannins, alkaloids, phenolics, triterpenoids, steroids, glycosides, and flavonoids can be found in West Indian elm (*Guazuma ulmifolia*) leaf extract. At a dose of 0.8 g/kgBW, West Indian elm leaf extract can slow the development of coronary artery atherosclerosis in Wistar rats (*Rattus norvegicus*) fed with a high-fat diet of a mix of duck egg yolks, flour, coconut oil, goat fat, and quail eggs. West Indian elm leaf extract is recommendable for individuals who frequently consume fatty foods to slow the development of coronary heart disease.

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DECLARATION OF INTERESTS

The authors have no conflict of interest.

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Nutrition Intake as a Risk Factor of Stunting in Children Aged 25–30 Months in Central Jakarta, Indonesia

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ABSTRACT

This study aims to determine the relationship between nutrient intake and the incidence of stunting and to determine the dominant factors associated with stunting in children aged 25–30 months in Gambir and Sawah Besar Districts, Central Jakarta, Indonesia. This research was a quantitative study with a cross-sectional study design. The type of data collected was secondary data from a case control study entitled Differences in Milk Intake and Stunting Incidence in Children Aged 25–30 Months in Central Jakarta in 2019. Data collected included height measurement, questionnaire-based interview, and 24-hour food recall. The percentage of stunting in children aged 25 to 30 months was 29.8%. The factor associated with stunting was iron intake (OR=5.0; 95% CI:1.02–25.25; p<0.05). Stunting was more likely to occur in children with inadequate iron intake than in those with adequate intake. To prevent stunting, iron-rich foods are essential and should be taken in sufficient amounts daily.

Keywords: children, iron intakes, nutrient, stunting

INTRODUCTION

Stunting is one of the indicators that determine child mortality and morbidity, including in developing countries. Globally, the prevalence of stunting in children under 5 years is 26%. WHO reports that 150–200 million children under the age of 5 are underweight and stunted (Verma & Prasad 2021). Stunting will reduce productivity as adults and cause a decrease in human resources always in the future (Sjarif *et al.* 2019). Childhood stunting has significant and long-term consequences for individuals, families, and countries (Muleta *et al.* 2021).

Indonesia is one of the five countries with the highest stunting rates in the world (Sjarif *et al.* 2019). Based on the 2021 Indonesian Nutritional Status Survey (SSGI), the prevalence of stunting in Indonesia is 24.4%. Central Jakarta is one of the areas with the highest prevalence of stunting in Jakarta, reaching 19.7% (MoH RI 2021). Gambir and Sawah Besar sub-districts are two sub-districts in Central Jakarta with a high frequency of stunting compared to other sub-districts (BPS 2019).

Stunting in children is mostly caused by poor nutrition, a high disease burden, inadequate child feeding, poor sanitation, and a lack of access to high-quality health and nutrition services (Muleta *et al.* 2021). For children's growth and development, an adequate intake of macro- and micronutrients (such as energy, protein, and vitamins A, C, and calcium) is important. As compared to those with sufficient intake (>90% RDA), children with energy and protein intake below 70% RDA have 1.3 times higher risk of stunting (Bening 2016).

Studies from Bogor demonstrate a strong correlation between children's insufficient carbohydrate consumption and the prevalence of stunting. Other research shows that fat intake is also a risk factor for stunting. Less of fat intake will have an effect on fat synthesis leading to an impact on children's growth (Basri *et al.* 2021; Wulandary & Sudiarti 2021).

Micronutrient consumption must also be factored in for children's growth. Stunting is a condition where a lack of vitamin C is thought to be a contributing factor. Vitamin C is essential for the production of collagen, a fiber and protein structure necessary for bone growth (Bening *et al.* 2016). Calcium intake is essential for the formation of bones. Calcium plays a role in the process of forming substrates and will

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accumulate during the formation of bone tissue. The mechanisms of cell division, growth, and tissue healing will be inhibited by the lack of calcium, which will have an impact on the weight and height of children (Srg *et al.* 2021).

Low iron intake can interfere with cognitive function and child growth because iron helps the immune system, making children less susceptible to disease. Inadequate zinc intake will also reduce the production and secretion of IGF-1 (growth factor), which might result in developmental delays in children (Losong & Adriani 2017). Children who are lack of zinc as well as vitamin A intakes are susceptible to stunting because vitamin A regulates protein synthesis.

A few years ago, the Indonesian government concentrated on sensitive nutritional interventions, which involved efforts aimed at reducing stunting rates through indirect causes of stunting. Ninety two percent of the total funds allocated for accelerating stunting reduction from 2019 to 2021 are utilized for sensitive nutritional interventions, such as providing access to drinking water, sanitation, nutrition, and parenting counseling (BAPPENAS 2021). Starting in 2022, the government is requesting cross-sectoral collaboration to enhance public education for eating goods derived from animals as strategy to improve nutrition and health. All of the necessary amino acids for growth are present in animal-derived protein. Having protein in a form that is easily absorbed by the body to support toddler growth, animal meals also include a variety of micronutrients, such as iron (Ernawati *et al.* 2016).

The objective of this research is to determine the association between micronutrient intake and the prevalance of stunting and to determine the most significant nutrients related with stunting in children aged 25–30 months in Gambir and Sawah Besar Districts of Central Jakarta.

METHODS

Design, location, and time

The method of cross-sectional research was selected in this study and secondary data were collected from a case-control study entitled "Differences in Milk Intake and Stunting Incidence in Children Aged 25–30 Months in Central Jakarta in 2019". The research was done in Central Jakarta from October 2018 to December 2019. Respondents in this research lived in the Gambir subdistrict (townships of Duri Pulo, Petojo Utara, Petojo Selatan, and Cideng) and in the Sawah Besar subdistrict (townships of Karang Anyar, Kartini, Mangga Dua Selatan, and Pasar Baru). This research obtained ethical clearance from the Ethics Commission of the Research and Community Service Institute at Atma Jaya University (Reference number: 1154/III/LPPMPM. 10.05/09/2019).

Sampling

The population in this study was 121 children aged 25–30 months. The age range was chosen because the age of 25–30 months old has the same food group, level of adequacy of energy and nutrients, and physical activity. The inclusion criteria in this research were mothers or caregivers who live in the Gambir and Sawah Besar Districts of Central Jakarta and had children aged 25 – 30 months, and mothers who were willing to be interviewed as research respondents.

The exclusion criteria were children aged 25–30 months with physical conditions that could affect the results of anthropometric measurements (limb and spinal disorders). All sampling units (121 children) were involved in the analysis of the relation between nutrition intake and nutritional status.

Data collection

Six enumerators from public health nutrition, Faculty of Public Health, University of Indonesia, were entrusted to collect the data. Anthropometric measurements were used to collect nutritional status data based on height for age and weight for age. Height measurements were taken in two trials to ensure the accuracy of the data. The instrument used was a calibrated microtoise from SECA 206 model.

Data on daily consumption intake and nutritional intake were obtained using the 24-hour food recall method, which was conducted over 2 days at the beginning of the study, and data on food consumption were converted to nutrient intake using the Nutrisurvey (Indonesian database). In this study the category of nutritional intake was less and good with a cut off point of 80% of the Indonesia's RDA table (2013).

Data analysis

All statistical analysis were done with SPSS version 17. The dependent variable in this study was stunting and the independent variables were macronutrient (energy, carbohydrate, protein, and fat) and micronutrient (calcium, iron, zinc, vitamin A, and vitamin C) intakes. Confounding variables in this study were the number of family members, family income, father's occupation, mother's occupation, father's education, mother's education, breast milk history, birth weight, RTIs history, diarrhea history, mother's nutrition literacy, and mother's knowledge level.The frequency distribution was tested in univariate analysis. A chi-square test with a level of significance of $p<0.05$ and a 95% CI was employed with bivariate analysis to examine the extent of the link between factors assumed to be connected to stunting.

Multiple logistic regression analysis was performed on variables arising from bivariate selection that had a $p<0.25$ or were regarded as significantly related to stunting, the variable were family income, mother's education, birth weight, energy intake, carbohydrate intake, protein intake, fat intake, calcium intake, iron intake, vitamin C intake, vitamin A intake, and zinc intake.

RESULTS AND DISCUSSION

Data from a total 121 respondents aged 25–30 months in Central Jakarta were successfully collected. The percentage of stunting in a study at selected locations in Central Jakarta in children aged 25 to 30 months was 29.8%. The percentage of stunted children in this research was higher than the prevalence of stunting in Indonesia, where the percentage according to SSGI (MoH RI 2021) was 24.4%. By 2025, the WHO aims to have lowered the prevalence of stunting among children by 40% (Verma & Prasad 2021).

According to bivariate analysis, stunting in children aged 25–30 months in Central Jakarta in 2019 was connected to birth weight and mother's education ($p<0.05$) (Table 1). In line with the findings of this study, Utami *et al.* (2018) reported that birth weight and birth length were risk factors for stunting in various Asian nations. Additional research indicated that Low Birth Weight (LBW) is the most frequent cause of stunting in children aged 12–23 months (Utami *et al.* 2018). According to the research findings, children with low birth weight are 3.8 times more probable to have stunting than children with normal birth weight. A child's low birth weight might be caused by a deficiency of nutritional fulfillment throughout pregnancy as well as the mother's bad health (Abbas *et al.* 2021).

According to prior research in Vietnam, a history of LBW was a potential factor for the occurrence of stunting in children under the age of three. It is claimed that babies born with LBW problems have an underdeveloped digestive tract, which reduces the digestive tract's ability to absorb fat and digest protein and as the result the body's nutrient reserves are insufficient. Infants born with low body weight often experience development stalling, which is compounded by inadequate or insufficient catch-up growth. Insufficient growth, faltering, and bad catch-up growth situations will result in stunting or conditions indicating an inability to achieve optimal growth in children (Kamilia 2019).

Table 1 shows that mothers with high degree of education are strongly associated with the occurrence of stunting ($p<0.05$). According to the study's findings, mothers with poor education had a 2.8 greater risk of having stunted children than mothers with higher levels of education. Parenting and feeding patterns in the first year of life play a significant role in child development, all of this is influenced by the educational background of the mother and has a significant impact on the quality of her upbringing (Apriluana & Fikawati 2018). In general, mothers with higher education will have a better understanding on health. More educated mothers are more aware of the importance of nutrition and serving their children appropriate meals for their growth and development (Chowdhury *et al.* 2020). Children of mothers with highest level of education below secondary school have a 2.4 times higher risk of stunting than children of mothers with highest level of education level above secondary school (Piniliw *et al.* 2021).

The following bivariate analysis will find out the link between nutritional intake and the prevalence of stunting (Table 2). Eight nutrients, including energy, carbohydrate, protein, fat, calcium, iron, vitamin A and vitamin C intakes appear to be significantly associated to the occurrence of stunting ($p<0.05$). The high ORs for macronutrient intake include energy, carbohydrate, protein, and fat intakes. Table 2 also shows micronutrient intakes with the highest OR being iron, vitamin C, calcium, vitamin A, and zinc intakes.

Table 1. Characteristics of children aged 25–30 months

Variables	Stunting n=36	Normal n=85	Total respondents n=121	OR	*p*
	n (%)				
Numbers of family members					
Large (>4 people)	10 (27.8)	26 (72.2)	36 (29.8)	Ref	0.927
Small (<4 people)	26 (30.6)	59 (69.4)	85 (70.2)	0.8 (0.3–2.0)	
Family income					
<Minimum salary level	26 (35.6)	47 (64.4)	73 (60.3)	Ref	0.124
>Minimum salary level	10 (20.8)	38 (79.2)	48 (39.7)	2.1 (0.9–4.8)	
Father's occupation					
Does not work	2 (40)	3 (60)	5 (4.1)	Ref	0.990
Working	34 (29.3)	85 (70.2)	116 (95.9)	1.0 (0.4–2.4)	
Mother's occupation					
Does not work	30 (31.3)	66 (68.8)	96 (79.3)	Ref	0.645
Working	6 (24)	19 (76)	25 (20.7)	1.4 (0.5–3.9)	
Father's education					
Low (<Junior high school)	12 (30.8)	27 (69.2)	39 (32.2)	Ref	1.000
High (> Senior high school)	24 (29.3)	58 (70.7)	82 (67.8)	1.1 (0.4–2.4)	
Mother's education					
Low (<Junior high school)	19 (44.2)	24 (55.8)	43 (35.5)	Ref	0.018*
High (>Senior high school)	17 (21.8)	61 (78.2)	78 (64.5)	2.8 (1.2–6.3)	
Breast milk history					
<6 Months	6 (31.6)	13 (68.4)	19 (15.7)	Ref	1.000
>6 Months	30 (29.4)	72 (70.6)	102 (84.3)	1.1 (0.3–3.1)	
Birth weight					
<3,000 g	21 (47.7)	23 (52.3)	44 (36.4)	Ref	0.002*
>3,000 g	15 (19.5)	62 (80.5)	77 (63.6)	3.8 (1.6–8.5)	
RTIs history					
Yes	9 (33.3)	18 (66.7)	27 (22.3)	Ref	0.824
No	27 (28.7)	67 (71.3)	94 (77.7)	1.2 (0.4–3.1)	
Diarrhea history					
Yes	6 (42.9)	8 (57.1)	14 (11.6)	Ref	0.350
No	30 (28)	77 (72)	107 (88.4)	1.9 (0.6–6.0)	
Mother's nutrition literacy					
Low (<51)	17 (34)	33 (66)	50 (41.3)	Ref	0.512
High (>51)	19 (26.8)	52 (73.2)	71 (58.7)	1.4 (0.6–3.0)	
Mother's knowledge level					
Low	14 (34.1)	27 (65.9)	41 (33.9)	Ref	0.584
High	22 (27.5)	58 (72.5)	80 (66.1)	1.4 (0.6–3.0)	

*Significantly related p<0.05 as analyzed with bivariate analysis
RTIs: Respiratory Tract Infections; OR: Odds Ratio

The children who are stunted consume a low quantity of nutrients, this is consistent with earlier research that showed macronutrient and micronutrient deficiencies were the most important factors influencing children's nutritional status in various nations (Verma & Prasad 2021). According to other studies, one of the main causes of stunting is a shortage of nutrients such as

Table 2. Characteristics of nutritional intake in children aged 25–30 months

Variables	Stunting n=36	Normal n=85	Total respondents n=121	OR	*p*
	n (%)				
Macronutrient					
Energy intake					
Less (<80% RDA)	34 (38.6)	54 (61.4)	88 (72.7)	Ref	0.001*
Good (>80% RDA)	2 (6.1)	31 (93.9)	33 (27.3)	9.8 (2.1–43.4)	
Carbohydrate intake					
Less (<80% RDA)	33 (37.5)	55 (62.5)	88 (72.7)	Ref	0.005*
Good (>80% RDA)	3 (9.1)	30 (90.0)	33 (27.3)	6.0 (1.6-21.2)	
Protein intake					
Less (<80% RDA)	11 (61.1)	7 (38.9)	18 (14.9)	Ref	0.004*
Good (>80% RDA)	25 (24.3)	78 (75.7)	103 (85.1)	4.9 (1.7–14)	
Fat intake					
Less (< 80% RDA)	28 (38.9)	44 (61.1)	72 (59.5)	Ref	0.014*
Good (> 80% RDA)	8 (16.3)	41 (83.7)	49 (40.5)	3.3 (1.3–7.9)	
Micronutrient					
Calcium intake					
Less (<80% RDA)	31 (36.9)	53 (63.1)	84 (69.4)	Ref	0.017*
Good (>80% RDA)	5 (13.5)	32 (86.5)	37 (30.6)	3.7 (1.3–10.6)	
Iron intake					
Less (<80% RDA)	34 (41.5)	48 (58.5)	82 (67.8)	Ref	0.0001*
Good (>80% RDA)	2 (5.1)	37 (94.9)	39 (32.2)	13.1 (2.9–58.0)	
Zinc intake					
Less (<80% RDA)	18 (40)	27 (60)	45 (37.2)	Ref	0.91
Good (>80% RDA)	18 (23.7)	58 (76.3)	76 (62.8)	2.1 (0.9–4.7)	
Vitamin A intake					
Less (<80% RDA)	22 (41.5)	31 (58.5)	53 (43.8)	Ref	0.022*
Good (>80% RDA)	14 (20.6)	54 (79.4)	68 (56.2)	2.7 (1.2–6.1)	
Vitamin C intake					
Less (<80% RDA)	31 (41.9)	43 (58.1)	74 (61.2)	Ref	0.001*
Good (>80% RDA)	5 (10.6)	42 (89.4)	47 (38.8)	6.0 (2.1–17.0)	

*Significantly related $p<0.05$ as analyzed with bivariate analysis
RDA: Recommended Dietary Allowances; OR: Odds Ratio

protein, energy, and iron, which are essential for growth and development of toddlers (Sholikhah & Dewi 2022). Children's food intake throughout the first thousand days of life, is strongly associated with brain development. Insufficient nutrition intake in children can contribute to poor motor development, low level of activity, and a lack of interest in the environment (Martiani *et al.* 2021).

The factors included in the multivariate analysis were family income, mother's education, birth weight, energy intake, carbohydrate intake, protein intake, fat intake, calcium intake, iron intake, vitamin C intake, vitamin A intake, and zinc intake ($p<0.25$). By performing the multiple logistic regression tests in the multivariate analysis, it was concluded that iron intake (OR=5.0; 95% CI:1.02–25.24) was the dominant factor in stunting. (Table 3). According to the study's findings, children with low iron consumption are at a higher possibility of stunting than children with good levels of iron. In this population, it was also discovered that 67.8% of children had less iron intake.

Previous research found that children who did not get enough iron had a 2.9 times higher risk of stunting than those who did. Another study in Surakarta revealed that children aged 1–3 years with iron deficiency were 3.2 times more probable to be stunted than children with adequate quantities of iron (Cahyati & Yuniastuti 2019). Iron is necessary for the development of bones, teeth, joints, muscles, and skin. Iron is involved in energy metabolism as well as the process of linear development and proliferation of body tissues (Cahyati & Yuniastuti 2019).

Iron deficiency can cause a child's growth to be stunted. Iron also plays a part in transporting oxygen to all tissues. If oxygenation of the tissues is interrupted, the bones will not grow adequately and the process of bone formation will be slowed down (Cahyati & Yuniastuti 2019; Nugraheni *et*

Table 3. Factors associated with stunting analysed with multiple logistic regression

Variables	OR (95% CI)	*p*
First model		
Family income	2.4 (0.89–6.70)	0.090
Mother's education	2.2 (0.63–7.64)	0.211
Birth weight	2.6 (0.86–7.89)	0.081
Energy intake	9.0 (0.65–124.76)	0.100
Carbohydrate intake	0.8 (0.77–9.04)	0.880
Protein intake	4.2 (1.07–16.46)	0.039
Fat intake	0.6 (0.17–2.46)	0.533
Calcium intake	4.0 (1.02–16.08)	0.046
Iron intake	11.24 (1.16–108.22)	0.036
Vitamin C intake	4.0 (1.02–16.08)	0.046
Vitamin A intake	0.7 (0.26–2.28)	0.647
Zinc intake	0.6 (0.18–1.96)	0.400
Final result		
Iron intake	5.0 (1.02–25.24)	0.047
Energy intake	4.6 (0.86–25.24)	0.074
Vitamin C intake	2.7 (0.82–9.13)	0.099

OR: Odds Ratio; CI: Confidence Interval

al. 2020a). According to Cahyati and Yuniastuti (2019), many stunting cases occur in children with a history of delayed bone growth.

In previous studies, low iron intake was caused by a lack of iron-containing foods. Red meat, chicken liver and cow liver are the finest sources of iron. Iron deficiency is more common in children who eat less iron-rich foods. The incorrect timing of weaning meals according to the child's age might also lead to the inadequate intake of iron (Nugraheni *et al.* 2020a). The findings of this study support the government's goal of promoting animal-based foods for toddlers to reduce stunting. Based on protein consumption per capita, Indonesia has met the national protein consumption adequacy guidelines; however, consumption of animal protein remains rather low. The consumption of eggs in Indonesia is between 4–5 kg/year, that of meat less than 40 g/person, and that of milk and its derivatives is 0–50 kg/person/year (Ministry of Health Republic of Indonesia (MoH RI 2023)). According to other studies, children who are malnourished consume a lot of protein from vegetables but less from animal proteins like fish and milk (Ernawati *et al.* 2016).

Normal children's protein intake was mostly from animal-source foods such as eggs, but the protein intake of stunted children was primarily sourced from legumes (Figure 1). Stunted children, in this population, consumed fewer iron-rich foods derived from animal protein, thus contributing to low iron intake. Animal proteins foods such as meat, fish, chicken, eggs and milk contain a relatively high level of protein (40% higher) compared to protein from vegetable sources (Ernawati *et al.* 2016). Animal proteins provide high levels of amino acids and can increase zinc and iron absorption, which can affect children's growth (Sholikhah & Dewi 2022).

Animal proteins provides amino acids that are necessary for the production of several hormones like thyroid hormone and Human Growth Hormone (HGH), which significantly improves the body's metabolic rate and promotes rapid growth and development (Sholikhah & Dewi 2022). Consuming foods containing animal protein is essential to get the required amounts of iron, which encourages faster growth and lowers stunting's prevalance in children.

Children with low energy intake are 4.6 times greater likely to be stunted than children with a high energy intake (Table 3). According to earlier studies on children aged 25–60 months in Lombok, children with less energy intake had 9.9 times greater risk of stunting than those with good energy intake (Anshori *et al.* 2020). Lack of energy intake will lead to stunted grow in children as a result of lack of nutrients in food. A lack of energy intake can be caused by an irregular eating pattern and a lack of food diversity. One of the factors affecting a child's growth is energy intake. A child's linear growth can be disrupted if the child experiences chronic energy deficiency (Nugraheni *et al.* 2020b).

This study also found that children with less vitamin C intake tend to have higher risk of stunting than children with sufficient vitamin C intake (Table 3). Vitamin C aids in the production of collagen, fiber, and protein structure, as well as

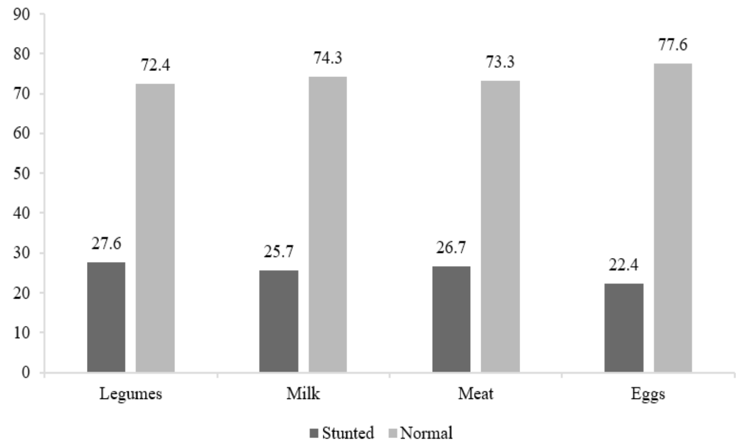

Figure 1. Percentage of children who consume iron-rich foods (n=121)

increasing the body's resistance against infections. Collagen is involved in the development of bones, teeth, and scar tissue and normal collagen cannot be generated in the absence of adequate quantities of vitamin C (Bening *et al.* 2016).

Children who are lacking in vitamin C will have difficulty in forming protein and collagen, which will inhibit their growth and development. A study on pregnant women in Egypt found that blood levels of vitamin C in pregnant women had a significant positive impact on neonatal anthropometry and placental weight. According to this study, other studies also showed that vitamin C had a significant correlation with an increased risk of stunting. Bening *et al.* (2016) discovered that children with insufficient vitamin C intake had a 2.9-fold higher risk of stunting than children with adequate vitamin C intake.

This study has several limitations, including a small sample size and an absence of an accurate description of the incident development process because it is a cross-sectional study. It is suggested that future studies should focus on large samples. According to the results presented in this study, attempts to improve iron consumption through food should be taken into consideration. Good iron consumption will influence behaviour and cognitive functions. Furthermore, iron helps produce hemoglobin, which promotes brain development. Several longitudinal studies have shown that nutrients, including macro- and micronutrients, promote an increase in children's linear growth.

CONCLUSION

The findings reveal that iron intake is the dominant factor associated with stunting (OR=5.0), after controlling for energy and vitamin C intakes. The study found that 67.8% of children had low intake of iron. The children in the stunting group ate more plant-based proteins than animal protein. Children who do not consume enough iron are more likely to be stunted than those who do. Iron consumption is crucial for preventing stunting and it needs to be met daily in sufficient amount.

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DECLARATION OF INTERESTS

The authors have no conflict of interest in in the conduct of this study from beginning to end

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Risk Factors of Subjective Cognitive Decline among Older People with Low Socioeconomic Status

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ABSTRACT

The aim of the study is to investigate the predictors associated with Subjective Cognitive Decline (SCD) especially among older people with low socioeconomic status. This was a cross- sectional study involving older people with low socioeconomic status in Kelantan, which is one of the poorest states in Malaysia. Data of anthropometry, body composition, cognitive function, sarcopenia, depressive symptoms, medical history, blood pressure and polypharmacy were obtained via face-to-face interview. SCD was determined by a single item in the Geriatric Depression Scale (GDS). Variables were analysed using the binary logistic regression model for identification of risk factors. A total of 293 older people with mean age of 69.1 years old was recruited. The SCD proportion in this study was 24.6%. One unit increase in Geriatric Depression Scale (GDS) score increases risk of subjective cognitive decline by odds of 1.814 (OR=0.595: 95% CI:1.441–2.283; p<0.001). Meanwhile, those with diabetes have a tendency of 2.972 to have SCD as compared to non-diabetics (OR=1.089: 95% CI:1.062–8.315; p<0.038). The prevalence of SCD in this study is high and may contribute to cognitive impairment. The predictors of SCD were larger waist circumference, having diabetes, and increasing score in GDS. SCD must be screened earlier and healthy lifestyle must be emphasized. Routine screening and monitoring of non-communicable disease risk factors are important for the prevention of SCD.

Keywords: cognitive impairment, depressive symptoms, socioeconomic status, subjective cognitive decline, older people

INTRODUCTION

Subjective Cognitive Decline (SCD) is a term indicating perception of worsening memory and frequent episodes of confusion for the past 12 months among individuals with normal cognition (Jessen *et al.* 2020). Although SCD is considered as subjective, it is one of the earliest manifestations of dementia that can affect the daily living of older people (Taylor *et al.* 2020). SCD is more common among older population as compared to younger people. Data obtained from the Behavioral Risk Factor Surveillance System found that SCD prevalence was higher among older people aged 65 years and above (11.7%) as compared to those within the age of 45–64 years old (10.8%). SCD was more prevalent among American older adults with at least one chronic disease such as stroke, heart diseases, chronic obstructive pulmonary diseases, kidney diseases and arthritis (Taylor *et al.* 2020). Findings from sixteen ageing cohort studies from 15 countries around the world demonstrated higher SCD among men than women, in lower education level, among Asian and Black Africans as compared to White people and in lower and middle-income countries as compared to higher income countries (Röhr *et al.* 2020). In addition, a meta-analysis of longitudinal studies has also shown that SCD increases risk of future cognitive impairment by 2.29-fold and a 2.16-fold excess risk for dementia (Wang *et al.* 2022).

Persistent worrying of having troubled cognition in SCD increase the likelihood of developing Alzheimer`s Disease (AD) in the future. Older people with SCD have 2.3 times higher risk of developing dementia and mild cognitive impairment due to higher exposure

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to oxidative stress (Lin *et al.* 2022; Dainy *et al.* 2018). Findings from a study by Schwarz *et al.* (2021) demonstrated that SCD severity is closely associated with regional cerebral beta-amyloid load (Schwarz *et al.* 2021). Role of socioeconomic status of an individual during childhood, across adulthood or in late-life towards cognitive function have been explored. Underprivileged socioeconomic status such as low parental socioeconomic status during childhood, low level of education, and limited income may lead to late-life cognitive performance due to structural brain changes (volume of hippocampus and frontal cortex) and functional brain activity (differing activation in the network of prefrontal, hippocampus and parietal working memory areas) (Künzi *et al.* 2021).

The Korean Community Health Survey found that SCD was reported by 17.4% of middle-aged adults and 29.4% of older adults; with older age, female and lower education as the risk factors of SCD (Roh *et al.* 2021). A cohort study comprising 1,165 cognitively normal older adults from the Chinese Alzheimer`s Biomarker and LifestyIE (CABLE) identified eight predictors of SCD namely older age, thyroid diseases, minimal anxiety symptoms, daytime dysfunction, female sex, anemia, sedentary lifestyle and loneliness (Wen *et al.* 2021). Lifestyle factors such as stress, depression, and sleep deprivation are also contributors to SCD (Miley-Akerstedt *et al.* 2019). Evidence also suggested that SCD is caused by psychological problems such as personality trait of neuroticism and family history of AD (Hill & Mogle 2018).

Malaysia, which is an ageing nation, is expected to face the problem of socioeconomic inequality among older adults due to limited savings and unemployment. The prevalence of older adults aged 65 years and older is 7.1% which is estimated to increase to 14.5% by year 2040 (Rosli *et al.* 2021). Low socioeconomic-status older adults are unable to meet their basic needs for establishing a decent standard of living such as proper clothing, good food, and comfortable house. Poverty among older adults is very high in Malaysia and issues related to social welfare will become very prominent (Shahar *et al.* 2019). A study conducted among 2004 older adults from the low socioeconomic status group demonstrated positive association between financial well-being and cognitive function (Foong *et al.* 2021). High socioeconomic status is related to better access to nutritious food, health care, and participation in mental and social demanding activities which reduces risk against cognitive decline (Nazri *et al.* 2021). A recent systematic review revealed that dementia among older people in Malaysia was reported to be 14.3% (Anuar *et al.* 2022). To our best knowledge, there is limited studies in Malaysia exploring the concept of subjective cognitive decline. As socioeconomic status is one of the contributors to SCD, it is essential to investigate the proportion of SCD among the low socioeconomic older adults as well as identify the possible predictors of SCD among older people.

METHODS

Design, location, and time

This is a cross-sectional study conducted among older adults with low-socioeconomic status in one of the poorest states in Malaysia, Kelantan. This study is a part of another study which investigated the predictors of sarcopenia among older adults in Kelantan. This study had been granted ethical approval from the organization`s ethics committee.

A total of 293 older adults aged 60 years and above participated in this study. Low socioeconomic status was defined as older people having education level of primary school and below and had household income below MYR3,030 (DOSM 2020). The exclusion criteria of the study were older adults with dementia (as diagnosed by doctors and reported by caregivers), those with physical limitations such as wheelchair bound or on crutches, and older adults with severe health problems such as cancer, end stage renal disease on haemodialysis, major depression, and psychiatric illness.

This study had been conducted during the Covid-19 lockdown period from October 2020 to May 2021 in five districts in Kelantan which was accessible and safe. Other districts in Kelantan were declared as red zones with high cases of Covid-19.

Sampling

Participants were able to provide written informed consent for participation in this study. Eligible participants were selected with the assistance of the village chief. Participants were contacted via telephone call or home visit. Trained interviewers conducted face-to-face interview using questionnaire. Proper social distancing with hand sanitation and temperature check were ensured throughout the data collection process. Data collection was conducted in small groups. A total of 293 older people were selected for this study The sample size had been calculated using the population prevalence formula, $n = \frac{z^2 p (1-p)}{d^2}$ z was the 95% confidence interval (1.96), p was the prevalence of older adults with muscle wasting in Kelantan (23.8%), d was the precision set at 5% (0.05).

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study has obtained ethical approval from the Human Research Ethics Committee Universiti Sains Malaysia (USM) (USM/JePeM/19070433). Informed consent has been obtained prior to recruitment of subjects.

Data collection

The data collection was conducted by the researcher with the assistance of enumerators in the field of nutrition via interview administered method.

Sociodemographic. Sociodemographic data consisted of sex, marital status, education level, household income, ethnicity, age, living status, employment status and smoking status.

Medical and falls history and supplement intake. This part includes questions regarding the presence of chronic diseases such as hypertension, high cholesterol, diabetes, heart diseases, stroke, kidney diseases, cancer, lung disease, arthritis, gastrointestinal diseases, urinary incontinence and other health problems. Medication intake of the subjects was recorded, and polypharmacy was defined as intake of five or more medications. Intake of vitamin, mineral or herbal supplement were recorded too.

Blood pressure. Blood pressure readings were measured twice using digital blood pressure monitoring machine. Readings were taken in relaxed condition to the nearest mmHg. If the systolic readings were above 140 mmHg or diastolic values were above 90 mmHg, subjects were asked to refer to a doctor for further consultation.

Anthropometry and body composition. Weight, height, body mass index, mid upper arm circumference, waist circumference, and hip circumference were recorded. Weight was measured to the nearest kg using Karada Scan Omron Body Composition Monitor (HBF-214). During weight measurement, subjects were asked to remove the items in the pockets and stand straight on the weighing scale.

Weight was recorded twice consecutively and the mean measurement was recorded for analysis. Meanwhile, height was measured to the nearest meter using the Seca stadiometer. Height measurement for subjects with scoliosis were assessed using arm span. Arm span was assessed as the distance between one middle finger`s tip to another middle finger`s tip. On the other hand, body mass index was calculated by dividing weight with square of height and categorized according to the cut-off point by Nutrition Screening Initiative (NSI) 1991 for older adults, namely underweight (less than 24 $kg/m^{2)}$, normal (24–27 kg/m^2) and overweight (>27 kg/m^2). Calf circumference is the widest section of the calf and was measured to the nearest cm in the sitting position with feet touching the ground. Furthermore, waist circumference, the assessment of abdominal obesity, was measured at the midpoint between the lowest last palpable rib and the top of the iliac crest. Hip circumference is the widest part of the pelvis and was measured to the nearest cm.

Body composition was analysis using the Karada Scan Omron Body Composition Monitor HBF-214 for assessing skeletal muscle mass and body fat percentage. Subjects were reminded to avoid caffeine, energy drinks and carbonated beverages 12 hours before examination, to take of sufficient quantity of water, to avoid exercising before examination, and to avoid wearing jewelleries or any metal-based ornaments during examination.

Sarcopenia status. Sarcopenia was assessed according to the revised Asian Work Group for Sarcopenia (AWGS) guidelines (Chen *et al.* 2020). A person was diagnosed with sarcopenia when they had low muscle mass and low muscle strength or low muscle performance. Severe sarcopenia was determined when an individual had poor score for muscle mass, muscle strength and muscle performance. Muscle strength was assessed using hand grip strength, while muscle mass was assed using Skeletal Muscle Index (SMI). SMI was calculated by dividing muscle mass with square of height. Low muscle mass was indicated by <7 kg/m² in males and <5.7 kg/m² in females (Fung *et al.* 2019). Poor hand grip strength was indicated by <28.0 kg for men and <18.0 kg for women. On the other hand, the short physical performance battery (SPPB) test was used for determining muscle performance. The total score of SPBB was the sum of the three tests with the score ranging from 0 to 12. Higher score indicated better muscle function especially at the lower extremity (Yasuda *et al.* 2017). Subjects with less or equal to nine points were considered having poor physical performance. Sarcopenia status was categorized into three categories namely normal, sarcopenia, and severe sarcopenia.

Cognitive function. The Malay version of Addenbrooke`s Cognitive Examination (ACE) III was used to assess cognitive function. It had good reliability with Cronbach alpha`s value of 0.829. ACEIII consisted of five domains of attention, memory, fluency, language and visuospatial. Score of ≤74 is categorized as risk of dementia (Kan *et al.* 2019).

Subjective cognitive decline. Subjective Cognitive Decline (SCD) was assessed using a single question from the Geriatric Depression Scale-15 (GDS 15) (item 10) which asked the subjects if they had memory decline. If subjects answered `yes`, this indicated the presence of subjective cognitive decline.

Depressive symptoms. Depressive symptom was determined using the short form 15-item Geriatric Depression Scale (GDS-15). GDS-15 had internal consistency value of 0.8 (Nyut *et al.* 2009). The final scoring for GDS was calculated by adding the scores for each individual answers. Presence of depressive symptoms were indicated by the score of 5 and above (Vanoh *et al.* 2016).

Data analysis

Data were analysed by using SPSS version 26.0. Normality testing was done based on histogram. For normally-distributed data, independent t-test was used whereas for non-normal distribution, Mann-Whitney test was used to test mean/median differences between categorical variable with two groups (presence of subjective cognitive decline) and numerical variable (age, education years, weight, body circumference, blood pressure). On the other hand, Chi-Square test has been employed to determine the association between two categorical variables. Predictors of subjective cognitive decline had been determined using binary logistic regression model adjusted for gender, education level, age, household income and Body Mass Index (BMI). Dependent variable was subjective cognitive decline with binary option (presence of depressive symptoms or none).

RESULTS AND DISCUSSION

Proportion of subjective cognitive decline (SCD) in this population was 24.6%. Sociodemographic parameters showed no significance difference between subjects with and without SCD (Table 1). Although not significant, findings showed that 50% of men and women in this study had SCD and 91.7% of those with SCD had household income lower than MYR900.

Proportion of SCD in this study was 24.6% and this is parallel with the findings of pooled study conducted among 39,387 older adults from 15 countries around the world which found prevalence of SCD between 25.1–26.1% (Röhr *et al.* 2020). In a Greek study comprising 1,456 older adults, 28.0% of the subjects responded positively when a single question about memory decline was asked (Vlachos *et al.* 2019). Varying prevalence rates are due to lack of standardization in the tools used for assessing SCD and the heterogeneity of the study population. Different studies used different approaches for assessing SCD. Some studies assessed SCD just by asking a single question of either open-ended, close-ended, or dichotomous, and some other studies target specific impairment related to domains such as naming, orientation, shopping, transportation, and calculation. The questionnaires used for assessing SCD have large variability. SCD must not be taken lightly as it is indeed a warning sign of neurodegenerative diseases. Patient who complained of SCD have higher risk of developing impaired cognition, and psychiatric issues such as depression, sleep disorders, daytime sleepiness, and forgetfulness (Wei *et al.* 2019).

Geriatric Depression Scale (GDS), which indicated depressive symptoms, showed significant differences among the subjects . Those with SCD had higher GDS score, which indicated

Table 1. Sociodemographic, lifestyle and sarcopenia status of subjects (n %) or median (IQR)

Parameters	Subjective cognitive decline		*p*
	Yes	No	
Gender			
Men	36 (50.0)	100 (45.2)	0.483
Women	36 (50.0)	121 (54.8)	
Education years, Median (IQR)	8.0 (6.0)	7.0 (8.0)	0.311
Household income			
<MYR900	66 (91.7)	202 (91.4)	0.944
MYR901–3,030	6 (8.3)	19 (8.6)	
Marital status			
Married	56 (77.8)	148 (67.0)	0.083
Unmarried/Widowed	16 (22.2)	73 (33.0)	
Smoking status			
Yes	17 (23.6)	47 (21.3)	0.676
No	55 (76.4)	174 (78.7)	
Sarcopenia status			
Normal	41 (56.9)	132 (59.7)	
Sarcopenia	18 (25.0)	47 (21.3)	0.803
Severe sarcopenia	13 (18.1)	42 (19.0)	
Polypharmacy			
Yes	2 (2.8)	2 (0.9)	0.234
No	70 (97.2)	219 (99.1)	

MYR: Malaysian Ringgit: IQR: Interquartile Range

presence of depressive symptoms, as compared to those without SCD (p<0.001). There were no significant differences in the association between SCD with anthropometry, blood pressure and cognitive function (Table 2).

Those with SCD reported significantly lower proportion of hypercholesterolemia (47.2%) and diabetes (63.9%), while high blood pressure was higher among those with SCD(62.5%) (p<0.05) (Table 3).

Table 2. Comparison and association between SCD and anthropometry, blood pressure and cognitive function median (IQR) or n (%)

Parameters	Subjective cognitive decline		*p*
	Yes	No	
Weight, kg, Median (IQR)	59.3 (19.1)	58.4 (16.2)	0.329
Hip circumference, cm, Median (IQR)	91.7 (14.0)	91.5 (11.8)	0.960
Waist circumference, cm Median (IQR)	86.0 (17.0)	82.0 (18.8)	0.194
Calf circumference, cm Median (IQR)	32.0 (5.5)	31.5 (4.5)	0.085
Systolic blood pressure, mmHg Median (IQR)	152.5 (33.0)	149.0 (35.0)	0.667
Diastolic blood pressure, mmHg, Median (IQR)	79.0 (15.0)	80.0 (15.0)	0.876
GDS score, Median (IQR)	5.0 (4.0)	3.0 (2.0)	$p<0.001^*$
Body mass index, n (%)			
Underweight	32 (44.4)	111 (50.2)	
Normal	19 (26.4)	48 (21.7)	0.633
Overweight	21 (29.2)	62 (28.1)	
Cognitive function, n (%)			
Normal	12 (16.7)	50 (22.6)	0.322
Impaired	60 (83.3)	171 (77.4)	

SCD: Subjective Cognitive Decline; IQR: Interquartile Range; GDS: Geriatric Depression Scale

Table 3. Association between subjective cognitive decline and comorbidities (n %)

Parameters	Subjective cognitive decline		*p*
	Yes	No	
Hypercholesterolemia			
Yes	25 (47.2)	41 (29.7)	0.023*
No	28 (52.8)	97 (77.6)	
High blood pressure			
Yes	45 (62.5)	103 (46.6)	0.019*
No	27 (37.5)	118 (53.4)	
Heart diseases			
Yes	2 (2.8)	16 (7.2)	0.171
No	70 (97.2)	205 (92.8)	
Diabetes			
Yes	26 (36.1)	43 (19.5)	0.004*
No	46 (63.9)	178 (80.5)	
Stroke			
Yes	1 (1.4)	5 (2.3)	0.649
No	71 (98.6)	216 (97.7)	
Renal diseases			
Yes	3 (4.2)	7 (3.2)	0.685
No	69 (95.8)	214 (96.8)	
Lung diseases			
Yes	0 (0.0)	5 (2.3)	0.198
No	72 (100.0)	216 (97.7)	
Arthritis			
Yes	15 (20.8)	48 (21.7)	0.874
No	57 (79.2)	173 (78.3)	
Gastrointestinal diseases			
Yes	12 (16.7)	29 (13.1)	0.451
No	60 (83.3)	192 (86.9)	
Incontinence			
Yes	10 (13.9)	15 (6.6)	0.061
No	62 (86.1)	206 (93.2)	

Table 4 depicts the predictors of subjective cognitive decline. Predictors of SCD based on the regression model are waist circumference, diabetes, and geriatric depression scale GDS. Greater waist circumference increases the odds of subjective cognitive decline by 1.059 (OR=0.057; 95% CI:1.005–1.116). Meanwhile, one unit increase in GDS score increases risk of SCD by odds of 1.814 (OR=0.595; 95% CI:1.441–2.283; p<0.001). Meanwhile, those with diabetes have a tendency of 2.972 to have subjective cognitive decline as compared to non-diabetics (OR=1.089; 95% CI: 1.062–8.315; p<0.038) (Table 4).

Multivariate regression model has revealed that the predictors of SCD are having greater waist circumference, higher score in GDS and having diabetes. Higher BMI and visceral adipose tissue are related with poor cognitive functioning. Central adiposity either at mid-life or at late-life is associated with greater rate of cognitive decline. Adiposity over the life course is associated with brain atrophy, white matter changes, and blood brain barrier integrity due to the presence of inflammatory markers and cytokines which may increase the risk of cognitive decline (West *et al.* 2017). Central adiposity is a risk factor of comorbidities such as hypertension, diabetes and hyperlipidemia which are also associated with dementia (Chen *et al.* 2022). Besides that, increased waist circumference is a component of metabolic syndrome which induces insulin resistance, hyperglycemia, dyslipidemia and hypertension. Insulin plays an important role in regulating neural metabolism and stimulates

Table 4. Predictors of subjective cognitive decline

Variables	B	SE	Exp (B)	95% CI for Exp (B)		*p*
				Lower	Upper	
Waist circumference	0.057	0.027	1.059	1.005	1.116	0.031*
Body mass index	-0.045	0.071	0.956	0.832	1.099	0.528
GDS score	0.595	0.117	1.814	1.441	2.283	0.000*
Systolic blood pressure	0.002	0.009	1.002	0.985	1.020	0.788
Cognitive function	-0.029	0.015	0.971	0.943	1.000	0.047*
High blood pressure	-0.371	0.527	0.690	0.246	1.940	0.482
High cholesterol	0.667	0.517	1.949	0.708	5.367	0.197
Diabetes	1.089	0.525	2.972	1.062	8.315	0.038*
Heart diseases	-1.740	1.220	0.176	0.016	1.920	0.154
Arthritis	1.214	0.722	3.366	0.818	13.850	0.093
Sarcopenia status						
Sarcopenia	0.027	0.541	0.960	1.027	2.968	0.356
Severe sarcopenia	-0.453	0.643	0.481	0.636	2.243	0.180

Model adjusted for age, gender, income, education years and smoking status

GDS: Geriatric Depression Scale; *Significant at $p<0.05$

glucose uptake in the regions of hippocampal and medial temporal lobe. Presence of insulin resistance in the hippocampus affects the spatial learning and synaptic plasticity (Hou *et al.* 2019). Women with metabolic syndrome were found to have poor scores in domains such as executive function and language (West *et al.* 2016).

Subjective cognitive decline has close relationship with diabetes mellitus. A recent systematic review has shown that type 2 diabetes mellitus or pre-diabetes is associated with cognitive impairment (Papunen *et al.* 2020). A population based cohort study consisting of 3363 participants from the Swedish National Study on Aging and Care-Kungsholmen found that poorly controlled blood glucose is associated with double risk of cognitive impairment and triple risk of cognitive impairment progressing to dementia (Dove *et al.* 2021). Chronic hyperglycaemia is associated with inflammation, oxidative stress and increased advanced glycation end products which contribute to accelerated cognitive decline. In addition, chronic elevation of blood glucose causes loss of endothelial cells, glial cells and neurons which are responsible for maintaining integrity of blood brain barrier (Marseglia *et al.* 2019).

Higher score in GDS, which indicated depressive symptoms, has been linked with SCD in the current study. Older adults with SCD and depression are vulnerable to developing cognitive impairment. Depression and SCD contributed to two important neurobiological pathways which increases risk of neurocognitive disorders. Monoaminergic system in the brain stem is linked with depression while cholinergic system in the basal forebrain is associated with SCD (Liew 2019). On the other hand, findings from the studies of neuroanatomical regions revealed that depression has been linked with atrophy of the entorhinal, and left middle frontal cortices, while SCD is associated with dysfunction of the left middle frontal cortices (Liew 2019). Systematic review has shown evidence from longitudinal studies that SCD was associated with depressive symptoms (Hill *et al.* 2016). In addition, relationship between SCD and depression still exist even after adjusting for sociodemographic characteristics and objective cognitive performance. Another study demonstrated that depression is associated with worsening of SCD-related outcomes such as needing help with daily activities, and disturbance in work and social activities. In fact, the same study found that link between depression and poorer SCD-related outcomes among younger age group of 45–69 years old (Brown *et al.* 2022).

The strength of this study is that it was conducted among low socioeconomic status older adults in one of the poorest states in Malaysia. Socioeconomic status, which comprised of education level, household income and occupation, has great influence on cognitive function of older adults. Underprivileged older adults may improve their mental wellbeing with proper social network and familial support (Tadai *et al.* 2023). Thus, stakeholders should identify strategies for increasing social capital for reducing socioeconomic inequality, increasing accessibility to health care services, and educating older adults on positive health-related behaviour. Moreover, this study used the interview-administered method for obtaining data for reducing bias. Meanwhile, the limitation of this study is that the results could not be generalized to the whole Malaysian population as it has been conducted in only one state in Malaysia and involving only one ethnic group.

CONCLUSION

In conclusion, the proportion of subjective cognitive decline among older adults with low socioeconomic status in this study was 24.6% which was comparable with other studies. Regression model revealed that the possible predictors of subjective cognitive decline were diabetes, higher GDS score and larger waist circumference indicating abdominal obesity. Previous literatures had highlighted that subjective cognitive decline increased the risk of dementia. Thus, subjective cognitive decline must be screened earlier, and healthy lifestyle must be emphasized.

RECOMMENDATIONS

Screening for SCD among older adults is essential. Proper management of chronic diseases such as maintaining optimal level of blood glucose or diabetes screening is essential for reducing risk of cognitive impairment. Furthermore, stress and depression among older adults can be managed with good social network, physical activity and access to psychological treatment. Abdominal obesity must be minimized with healthy diet and physical activity. Thus, future studies can focus on the effectiveness of these intervention programs as well as investigate the changes in biomarkers among those with subjective cognitive decline.

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DECLARATION OF INTERESTS

All authors have no competing interest to declare that are relevant to the content of this article.

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INDEX OF AUTHORS

INDEX OF SUBJECTS

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