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Correlation between Blood Glucose Level and Short-Term Memory Score among 4th and 5th Grades of Primary School Children in Bogor, Indonesia

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ABSTRACT

The study aimed to analyze the correlation between Blood Glucose levels (BG) and Short-Term Memory scores (STM) among primary school children in Bogor, as controlled by Socioeconomic Status (SES), BMI-for-age (BAZ), Hemoglobin (Hb), folate, and vitamin B12 levels. It was a cross-sectional study applied for 915 students of the 4th–5th grades from 16 primary schools in the suburban area of the Cijeruk district. Morning blood samples were drawn from venous puncture of the inner arm. The specimens were then transported to laboratories to analyze the BG (hexokinase methods), Hb (non-cyanide Hb), as well as folate, and vitamin B12 using Liquid Chromatography and Mass Spectrometry. The STM was obtained through an object recall test performed by trained psychologists. The actual body weight and height were measured to determine BAZ. The characteristics of subjects and their SES were collected through interviews and structured questionnaires. Mann-Whitney and Chi-square tests were performed to compare differences between variables that were grouped by genders. Correlations between predictors and predicted variables were analyzed using simple logistic regression for the bivariates analysis and a binary logistic regression test for the multivariate analysis. There were significant differences in BAZ, BG, and STM between boys and girls ($p<0.05$). Most of the subject's BAZ was normal (85.2%). About 50.9% of subjects had normal Hb, 99.8% had folate deficiency, 47.8% had normal vitamin B12 level, and 54.9% had good STM score. There was no significant correlation between BAZ, Hb, folate, and vitamin B12 level with STM ($p\geq0.05$), respectively. There was a significant positive correlation between BG and STM ($p<0.05$; OR=1.583; 95% CI:1.067−2.348) after being controlled by BAZ, subject's characteristic, and SES. In conclusion, optimum blood glucose level improved the STM in primary school children. Therefore, provision of balanced diet, more especially breakfast, for school children is highly important.

Keywords: blood sugar, cognition, nutritional status, school-age children, socioeconomics

INTRODUCTION

Nutrition affects both the development and health of brain structure and function. It provides proper components to create and maintain brain function that is critical for improved cognition and academic performance (Burkhalter & Hillman 2011). Investigation on nutrition related to cognitive performance is a challenge where cognitive performance may result from many factors (not only nutrition) such as demographics and socioeconomics (Lipina & Sigretin 2015), as well as genetics (Robinson *et al.* 2015) that make the effects difficult to determine. Regardless, understanding the role of nutrition is important as nutrition is one of the modifiable factors that can optimize cognitive performance.

Glucose is a macronutrient that is perhaps the most thoroughly studied in terms of its effects on cognitive function (Sünram-Lea & Owen 2017). Glucose is the primary fuel to produce energy for the brain (Nimgampalle *et al.* 2021). Acute changes in glucose supply have been found to affect cognitive function and a review study found that glucose affected cognitive performance, specifically in the memory domains (Smith *et al.* 2011).

Glucose metabolic rate of the brain is different and varies across the life span. Initially, the rising glucose consumption rates occur from newborns until they reach about four years old. Large amounts of glucose are also required between four to ten years old because an intense learning process occurs during this period, where

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children learn a lot related to basic cognitive concepts, such as reading, writing, and arithmetic (Sunehag & Haymond 2004). Oxygen is required for aerobic metabolism to utilize the brain energy and hemoglobin plays an essential role as the carrier of oxygen transferring (Kustiyah *et al.* 2005). On the other hand, folate and vitamin B12 are commonly mentioned as critical for the brain's optimal development and neurological function throughout the lifecycle (Van De Rest *et al.* 2012).

Socioeconomic Status (SES) is another factor that is strongly found to influence cognitive performance (such as memory), especially in children (Lipina & Sigretin 2015).

Previous study by Kustiyah *et al.* (2005) found that blood glucose levels are positively associated with STM in elementary school children. However, this study has not discussed the relationship between blood glucose levels and STM when analyzed simultaneously with other factors (such as hemoglobin, folate, and vitamin B12 levels, nutritional status, ages, genders, and SES) often associated with children's memory outcomes. It is crucial to analyze them simultaneously as covariates, given that memory performance is the result of interaction between various factors.

Understanding the role of nutrition-related cognition (in memory domains) in primary school children is essential. Based on Piaget's third stage of cognitive development in children 7−12 years old, a growth spurt occurs in the brain, especially in the frontal lobes that are responsible for cognitive function, with the growth peaks at about 8−10 years old (Ibda 2015; Shaffer & Kipp 2013). The optimum cognitive development in this period will affect their quality of life in the future, including achievements in career, their health, and also mortality risk. Therefore, the purpose of this study was to analyze the correlation between blood glucose level and STM score in primary school children in Bogor as adjusted by hemoglobin, folate, and vitamin B12 levels, nutritional status, ages, genders, parental educational levels, and family income.

METHODS

Design, location, and time

Our study was observational research with a cross-sectional study design, located in the suburban district of Cijeruk, Bogor in 16 public elementary schools. Where previous studies focused on urban or rural areas (as Cijeruk is a sub-urban area), no similar research has been conducted in the area before. The data collection was carried out from October to December 2019.

Sampling

A total of 915 healthy children (boys and girls) from fourth and fifth grade (ranging in age eight to 13 years) were enrolled in our study based on the inclusion criteria and ability to pass the writing and reading test. The inclusion criteria for the subject were based on several cognitive screening test including logical thinking, ability to engage in more complex cognitive tasks (Piaget's theory), and having good reading and writing ability. In addition, they should also be able to communicate well with teachers, parents, and researchers. Only students who passed the screening test and other criterias set by the researcher were recruited, thus the sampling method was purposive. Those who agreed to participate signed the informed consent and assent were examined for their blood profiles and were included in all the data collection. On the other hand, the exclusion criteria were last year's students (6^{th} grade; because of their final exams preparation) and the third or lower grade (had limited ability to read, write, and communicate well). Transfer students (within the previous six months) suffering from a severe illness, physically or mentally disabled, and girls who have menstruated were also excluded from our study.

Slovin's formula for population determined the total subject of the study. The population (N) was 9,241 of the public primary school students in the Cijeruk district (BPS-Statisticts of Bogor Regency 2019), and the margin of error used was 5%.

Data collection

The study was approved by The Ethics Commission of IPB University, with record number 242/IT3.KEPMSM-IPB/SK/2019. Morning blood samples were drawn in venous puncture of the inner arm, done by trained physicians. Subjects were only allowed to drink plain water (fasting from foods) from the night before bed until the blood draw. The specimens were then transported to certified laboratories at

Prodia Lab of Bogor (to analyze blood glucose and hemoglobin) and Labkesda of Bogor (folate and vitamin B12 analysis). In vitro blood samples were analyzed using hexokinase methods (blood glucose assay), non-cyanide hemoglobin (hemoglobin), and Liquid Chromatography, and Mass Spectrometry (folate and vitamin B12).

Anthropometric data includes actual body weight and height were measured using weight scales and microtoise (minimum reading scale of 0.1 cm), respectively. Then, the Body Mass Index (BMI) for age was determined. An object recall test was used to obtain the scores of Short-Term Memory (STM), done by trained psychologists. The use of the object recall test refers to the previous studies conducted by Kustiyah *et al.* (2005) and Lubis *et al.* (2008), where this method is relatively simple and comparatively inexpensive. A set of pictures was shown to the subjects, and they had to memorize them in 60 seconds. Then they were asked to list as many pictures as they could remember. The score was calculated based on the number of correct answers. The characteristic of subjects and the SES of the family were collected through interviews and structured questionnaires.

Data analysis

Data analysis involved calculating and describing the prevalence of blood glucose, hemoglobin, folate, vitamin B12, BMI for age, STM scores, subject's characteristic, and SES. The serum concentrations were presented in mg/dl for blood glucose, g/dl for hemoglobin, ng/ml for folate, and pg/ml for vitamin B12. Blood glucose levels ranged from 70−110 mg/dl was considered normal, less than 70 mg/dl was low, and higher than 110 mg/dl was high (American Diabetes Association 2015). The cut-off point of normal Hb level was >11.4 g/dl, Hb levels ranged from 11−11.4 g/dl was determined as mild anemia, 8−10.9 g/dl as moderate anemia, and <8.0 g/dl was considered as severe anemia (WHO 2011). Folate serum was categorized as normal (≥5.0 ng/ml), possible deficiency (2.5−4.9 ng/ml), and deficiency (<2.5 ng/ml). Vitamin B12 serum was categorized as normal (≥200 pg/ml) and the deficiency (<200 pg/ml) (Bozkaya *et al.* 2017). Furthermore, the subject's STM was classified as "good" if the score was more than the median value and vice versa. Mann-Whitney and Chi-square tests were performed to compare differences between boys and girls for each variable.

In this study, BAZ Hb level, folate, vitamin B12 serum, and SES was classified as the covariates. We performed simple logistic regression test to analyse the correlation between blood glucose and the covariates to STM score in bivariate (crude analysis).

A binary logistic regression test was run to evaluate the simultaneous correlation between variables with a $p<0.250$ (Hosmer *et al.* 2013) in bivariate analysis to the STM score. If the p-value of the output was less than 0.05 ($p<0.05$) is considered significantly correlated. The confounding test was carried out by measuring the association both before and after adjusting for a potential confounder variable. If the difference between the association is more than the equivalent 10%, then the confounder was present, and vice versa (LaMorte & Sullivan 2021). No confounder found in our study (analytical data not presented in this manuscript).

RESULTS AND DISCUSSION

Subject's characteristics and socioeconomic status

Data on subjects' characteristics and SES from 915 students grouped by genders are presented in Table 1. There were significant differences between boys and girls both in age and pocket money ($p<0.05$) but there was no difference in joining tutoring class ($p\geq0.05$).

Table 1 also shows that most of the subjects had low parental educational levels (did not graduate from Senior high school), both in father (81.5%) and mother (89.3%). Furthermore, the family income of most subjects (58.3%) was also low (<IDR1,500,000 per month).

Nutritional status, blood biomarkers, and short-term memory scores of subjects

Table 2 shows that there was a significant difference in BMI between boys and girls ($p<0.05$), which girls had a higher score of BMI.

Most subjects had normal blood glucose levels (89.9%), where boys showed a higher mean blood glucose concentration than girls ($p<0.05$). About 50.9% of subjects had a normal level of hemoglobin, while 40.1% were anemic. The study found that the prevalence of anemia among boys was higher than girls, where 52.2% of boys

Table 1. Mean and disitribution of subjects' characteristics based on genders

Variables	Boys (n=446)		Girls (n=469)		Total (n=915)		p
	n	%	n	%	n	%	
Subject's age (years old)							
Mean±SD	10.72±0.84		10.50±0.77		10.60±0.81		0.000[b*]
Median (min−max)	10.80 (8.6−13.1)		10.45 (8.4−13.3)		10.58 (8.4−13.3)		
Taking after-school tutoring programs							
Yes	81	18.2	89	19.0	170	18.7	0.751[a]
No	365	81.8	380	81.0	745	81.3	
Pocket money (IDR/day)							
Mean±SD	6,130±3,560		6,747±3,534		6,447±3,558		0.001[b*]
Median (min−max)	5,000 (0−30,000)		5,000 (0−25,000)		5,000 (0−30,000)		
Father's age (years old)							
Mean±SD	42.82±7.80		42.21±8.05		42.51±7.93		0.151[b]
Median (min−max)	42 (29−75)		40 (25−72)		41 (25−75)		
Mother's age (years old)							
Mean±SD	37.33 ±6.82		36.25 ±6.58		36.77±6.71		0.019[b*]
Median (min−max)	37 (25−62)		35 (22−55)		36 (22−62)		
Father's educational level							
No formal education	63	14.1	79	16.8	142	15.5	0.294[a]
Elementary school	221	49.6	209	44.6	430	47.0	
Junior high school	89	19.9	85	18.1	174	19.0	
Senior high school & college	73	16.4	96	20.5	169	18.5	
Mother's educational level							
No formal education	77	17.3	89	19.0	166	18.1	0.927[a]
Elementary school	229	51.4	243	51.8	472	51.6	
Junior high school	89	19.9	90	19.2	179	19.6	
Senior high school & college	51	11.4	47	10.0	98	10.7	
Family income (IDR/month)							
<1,500,000	272	61.0	261	55.7	533	58.3	0.066[a]
1,500,000−2,500,000	101	22.7	128	27.2	229	25.0	
2,500,000−3,500,000	50	11.2	40	8.5	90	9.8	
>3,500,000	23	5.1	40	8.6	63	6.9	

[a]Chi-square test; [b] Mann Whitney test; IDR: Indonesian Rupiah

and 46.1% of girls were anemic. Nevertheless, there was no difference in mean hemoglobin levels between boys and girls.

Regarding serum folate, this study found that almost all subjects were folate-deficient (99.8% from total subjects), and about 55.2% of total subjects were deficient in vitamin B12. The statistical analysis also did not find the difference in folate and vitamin B12 serums between boys and girls, respectively. The deficiency of folate and Vit B12 was much more common in many countries because of many factors, but insufficient dietary intake was assumed to be the primary reason (Khan & Jialal 2021). The Object recall task assessment was used to measure STM performance and found a significant difference between boys and girls, where girls had higher scores than boys (Table 2).

Tabel 2. Body mass index, blood biomarkers, and short-term memory scores of subjects

Variables	Boys (n=446)		Girls (n=469)		Total (n=915)		p
	n	%	n	%	n	%	
Body mass index (kg/m^2)							
Mean±SD	16.56±2.85		17.05±3.17		16.81±3.02		0.007[a*]
Median (min−max)	15.98 (12.2−33.5)		16.29 (12.13−34.3)		16.09 (12.1−34.3)		
Body mass index for age							
Severe thinness	1	0.2	0	0	1	0.1	0.111[b]
Thinness	14	3.1	7	1.5	21	2.3	
Normal	381	85.4	399	85.1	780	85.2	
Overweight	22	4.9	30	6.4	52	5.7	
Obese	28	6.3	33	7	61	6.7	
Blood glucose (mg/dl)							
Mean±SD	11.57±0.99		11.65±0.93		11.61±0.96		0.595[a]
Median (min−max)	11.6 (7.2−15.1)		11.6 (8.4−14.6)		11.6 (7.2−15.1)		
Low (<70)	37	8.3	51	10.9	88	9.6	
Normal (70−110)	406	91	417	88.9	823	89.9	
High (>110)	3	0.7	1	0.2	4	0.4	
Serum hemoglobin (g/dl)							
Mean±SD	11.57±0.99		11.65±0.93		11.61±0.96		0.595[a]
Median (min−max)	11.6 (7.2−15.1)		11.6 (8.4−14.6)		11.6 (7.2−15.1)		
Severe anemia (<8.0)	2	0.4	0	0	2	0.2	
Moderate (8.0−10.9)	90	20.2	97	20.7	187	20.4	
Mild (11−11.4)	141	31.6	119	25.4	260	28.4	
Normal (>11.4)	213	47.8	253	53.9	466	50.9	
Serum folate (ng/ml)							
Mean±SD	0.45±0.40		0.45±0.35		0.45±0.37		0.595[a]
Median (min−max)	0.33 (0.01−3.49)		0.35 (0.01−2.15)		0.34 (0.01−3.49)		
Deficiency (<2.5)	444	99.6	469	100	913	99.8	
Possible deficiency (2.6−5.0)	2	0.4	0.0	0.0	2	0.2	
Normal (≥5.0)	0	0	0	0	0	0	
Serum vitamin B12 (pg/ml)							
Mean±SD	236.18±181.72		249.08±220.11		242.79±202.30		0.511[a]
Median (min−max)	200 (80−3,220)		200 (80−3,440)		200 (80−3,440)		
Deficiency (<200)	236	52.9	242	51.6	478	52.2	
Normal (≥200)	210	47.1	227	48.4	437	47.8	
Short-term memory score							
Mean±SD	54.83±21.17		61.63±19.83		58.32±20.76		0.000 a[*]
Median (min−max)	53 (7−100)		60 (7−100)		60 (7−100)		
Low	234	52.5	179	38.2	413	45.1	
Good	212	47.5	290	31.7	502	54.9	

[a]Mann Whitney test; [b]Chi-square test

Correlation between variables to short-term memory

Table 3 shows that blood glucose level correlated significantly with STM score. In contrast, BMI for age had no association with STM score. This finding was in line with Ong *et al.* (2010) and Veldwijk *et al.* (2011). Furthermore, Haile *et al.* (2016) explained that BMI-for-age indicated the acute nutritional status, and it did not determine or impair the cognitive function.

The bivariate analysis showed that folate and vitamin B12 serum did not correlate with STM. Our result was inline with Kvestad *et al.* (2020) and Rauh-Pfeiffer *et al.* (2014). Furthermore, Van der Zwaluw *et al.* (2014) explained that vitamin levels could be linked to changes in the brain but not translated to psychological tests in healthy people.

Table 3 also shows that hemoglobin level had no relation with STM. Anemia was associated with lower cognitive function in specific domains, but not for memory (Schneider *et al.* 2015).

A binary logistic regression test was performed to determine the combination of factors that best predict STM scores in children. After being controlled by the covariates, blood glucose level was still found to be correlated with STM ($p<0.05$; OR=1.583; 95% CI:1.067−2.348) (Table 4). It can be interpreted that blood glucose within normal levels has the opportunity by 1.583 times to increase the STM score of the primary school children compared to those who abnormal's.

Kustiyah *et al.* (2005) found similar results with this study, where blood glucose level was positively correlated with STM in primary school children. The human brain is an organ that is much more energetically demanding than other organs, and glucose is the primary energy source for the brain. Several specific neurocognitive mechanisms thought potentially underlie the effects of glucose in the brain in its relation to memory outcomes. However, the most robust theory in terms of empirical evidence is the hypothesis that glucose improves memory via its effects on the synthesis of Acetylcholine (ACh). In addition, there is also a role for insulin, ATP, and extracellular glucose availability in the brain on memory (Smith *et al.* 2011).

Oral glucose consumption or acute stress/emotional arousal will increase the concentration of glucose circulating in the periphery and

Table 3. Output of bivariate analysis between variables and short-term memory score

Variables	Short-term memory (reference=1)		
	B	Sig.	OR (95% CI)
BMI-for-age			
Normal (1) / Abnormal (0)	0.247	0.186	1.280 (0.888−1.845)
Serum hemoglobin			
Normal (1) / Abnormal (0)	0.012	0.927	1.012 (0.780−1.313)
Blood glucose level (mg/dl)			
Normal (1) / Abnormal (0)	0.492	0.012*	1.635 (1.114−2.400)
Serum folate (ng/ml)			
>2.5 (1) / ≤2.5 (0)	-0.196	0.890	0.822 (0.051−13.188)
Serum vitamin B12 (pg/ml)			
>200 (1) / ≤200 (0)	0.093	0.485	1.097 (0.845−1.424)
Gender			
Boy (0) / Girl (1)	0.581	0.000*	1.788 (1.374−2.327)
Subject's age (years old)			
>11 (1) / ≤11 (0)	0.363	0.008*	1.438 (1.099−1.881)
Taking after-school tutoring programs			
Yes (1) / No (0)	0.336	0.054	1.399 (0.994−1.969)
Pocket money (IDR/day)			
≥5,000 (1) / <5,000 (0)	0.418	0.016*	1.519 (1.081−2.136)
Father's educational level			
High (1) / Low (0)	0.397	0.024*	1.487 (1.055−2.096)
Mother's educational level			
High (1) / Low (0)	0.389	0.078	1.476 (0.957−2.275)
Family income (IDR/month)			
≥1,500,000 (1) / <1,500,000 (0)	0.318	0.019*	1.374 (1.053−1.792)

Bivariate analysis; Simple logistic regression test; B: Constant-Coefficient; Sig: Significant Value; OR: Odds Ratio; CI: Confidence Interval; IDR: Indonesian Rupiah; BMI: Body Mass Index then distribute to the central nervous system. Glucose increases the synthesis of ACh in the hippocampus, brain insulin secretion, intra-neural ATP levels (production of neurotransmitters), and the availability of extracellular glucose in the brain (to supply the glucose needs for the hippocampus); which in turn acts as a potential mediator of memory enhancement (Smith *et al*. 2011).

Based on the logistic regression test (Table 4), it shows that the value of the Odds Ratio (OR) for sex is higher (OR=1.896; reference value was one for girl) than the OR for blood glucose (1.583). Hence, it is assumed that girls' memory scores are better than the boys' even though boys' blood glucose levels are higher than the girls' (Table 2). In general, women had more significant amounts of estrogen in the dorsolateral prefrontal cortex and the hippocampus, to which these two brain regions are associated closely related to memory (Slotnick 2017). Asperholm *et al.* (2019) show that females in general, have a higher tendency in memory performance, especially in object recall memory (Spets & Slotnick 2019) compared to males. In line with the literature studies, girls' memory was better than boys', measured by remembering pictures or objects. Podila (2019) stated that girls are more motivated than boys to perform well during elementary school. It is also thought to be a contributing factor that causes girls to have better memory scores than boys.

In this study, the subject's ages varied from 8 years to 13 years. Table 4 also shows that the older subject's age, the better the STM score ($p<0.05$; OR=1.567; 95% CI:1.183−2.076). Brenhouse & Andersen (2011) state that cognitive function continues to develop with age, as reflected from the maturation process of the central nervous system. The process continues to occur over time, not only in childhood but also until humans reach adulthood.

As mentioned above in Table 4, factors that were positively associated with STM were blood glucose level, gender, and age ($p<0.05$). People with higher glucose metabolism (related to glucose facilitation effect) performed better on cognitive tasks than those who had low glucose level. Roalf *et al.* (2014) found that older children (late childhood) had a higher brain glucose metabolism than younger ones due to brain maturation. Gur & Gur (2017) also found that brain glucose metabolism was generally higher in girls than boys during this period. Specifically, girls had significantly higher glucose metabolism in the brain regions responsible for memory tasks (hippocampus). While in boys, it performed better in spatial and motoric tasks (Gur & Gur 2017).

The result of regression analysis in SES are also shown in Table 4. The significant associations were found when SES, consisting of parental education, pocket money, and family income

Table 4. The final model of variables that predict the short-term memory score

Variables	Short-term memory (reference=1)		
	B	Sig.	OR (95% CI)
BMI-for-age			
Normal (1) Abnormal (0)	0.229	0.234	1.258 (0.862−1.834)
Blood glucose level			
Normal (1) Abnormal (0)	0.459	0.022*	1.583 (1.067−2.348)
Gender			
Boy (0) Girl (1)	0.639	0.000*	1.896 (1.443−2.490)
Subject's age (years old)			
>11 (1) ≤11 (0)	0.449	0.002*	1.567 (1.183−2.076)
Taking after-school tutoring programs			
Yes (1) No (0)	0.238	0.187	1.268 (0.891−1.805)
Pocket money (IDR/day)			
≥5,000 (1) <5,000 (0)	0.344	0.055	1.411 (0.992−2.008)
Father's educational level			
High (1) Low (0)	0.252	0.221	1.287 (0.859−1.928)
Mother's educational level			
High (1) Low (0)	0.216	0.406	1.241 (0.746−2.063)
Family income (IDR/month)			
≥1,500,000 (1) <1,500,000 (0)	0.177	0.224	1.193 (0.898−1.586)

Multivariate analysis, binary regression logistic test; B: constant-coefficient; Sig: Significant Value: OR: Odds Ratio; CI: Confidence Interval; BMI: Body Mass Index: IDR; Indonesian Rupiah independently analyzed with STM (bivariate analysis shown in Table 3). Nevertheless, when they simultaneously analyzed with other variables (BMI-for ages, subject's ages, and genders) by logistic regression test, the result was found to be not significant.

Piccolo *et al.* (2016) pointed out that age moderates the effects of SES on cognitive performance (including memory domains). The impact of SES on cognitive performance is only prominent until the age of 9 years, while after that, the effect decreases. The influence of the environment and peers becomes more substantial than the socioeconomic influence of the family in the period of age. In addition, the thickness of the brain's cortex also increases and improves cognitive performance. The cortex is continuously developed until the adolescence period is started (Piccolo *et al.* 2016). Therefore, it can be assumed that age reduces the effect of family SES on the subject in this study (Table 4).

Although gender and age affect the subject's memory score, the blood glucose level is a factor that is modifiable in terms of achieving a more optimal memory performance. However, further studies are essential to be conducted regarding nutrition and gender on memory performance. Our study implies that optimal blood glucose levels are essential for elementary school children to optimize their memory performance during school. One way to keep the blood glucose level optimal is having healthy breakfast regularly before school. Various studies have shown a strong relationship between breakfast habits and blood glucose levels in school children (Tang *et al.* 2017). Some limitations deserve attention in future studies. Firstly, no measurement of stress level and motivation is carried out in this study to influence cognitive performance. In addition, the instrument of memory test measurement was only for remembering pictures that could not be enough to describe the STM of all subjects however it is universal enough for cross culture use. Besides the limitations, this study also had strengths where a large sample size was collected, a direct measurement for short-term memory with a universal tools conducted by trained professional was used, and many covariates were adjusted. We also presented the many nutritional biomarkers that may contribute to cognitive ability, which are still rarely found in Indonesian research related to STM among school children.

Further research is needed to deepen the study results and clarify the influence of nutritional factors that can affect the subject's memory ability.

Furthermore, the test instrument used to measure STM scores can be made more varied (not only by remembering pictures) to describe the memory abilities of school-age children in more detail.

CONCLUSION

Blood glucose levels were positively correlated to STM scores ($p<0.05$) with an odds ratio of 1.583 (95% CI:1.067−2.348) after being controlled by BMI-for age, subject's age, gender, tutoring after class, pocket money, parents' educational level, and family income among primary school children grade 4th and 5th. Students with normal blood glucose level have 1.583 times opportunity to have better short-term memory score compared to those who have low blood glucose level.

Suggestion for future research include utilizing a more robust design (Randomized control trial) as well as adding other variables such as the subject's motivation in learning and stress level. The authors also suggest parents to provide regular breakfast for their children to keep their blood glucose at a normal level when participating in school activities. In addition, parents are also expected to provide balanced and varied nutritious food every day so that the children's nutritional needs are met to maintain health and optimize their overall cognitive performance.

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DECLARATION OF INTERESTS

All authors have no conflict of interest in the research.

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Higher Parental Age and Lower Educational Level are Associated with Underweight among Preschool Children in Terengganu, Malaysia

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ABSTRACT

This cross-sectional study aimed to investigate the determinants of underweight among preschool children. A total of 218 preschool children were enrolled. Their sociodemographic data were collected using self-reported questionnaires whilst body weight and height were measured, recorded and the BMI for-age z-score was calculated using WHO AnthroPlus software. Of all preschool children participants, 47.7% were male and 53.3% were female. Most of them were Malays (99.5%), aged 4 to <5 years (40.8%) and came from low-income household (92.7%). Overall, the prevalence of underweight, normal, overweight and obese was 17.9%, 73.8%, 4.6% and 3.7% respectively. The underweight prevalence was higher than the national prevalence (13.7%). Of all parent participants, 12.4% were male and 87.6% were female. Most of them aged 30−39 years (55.5%) and did not hold a degree (89.4%). Multivariate logistic regression showed that parental age and their level of education were the determinants of childhood underweight. The risk of being underweight increased with the age of parents (p=0.033) and lower level of education of parents (p=0.042). In conclusion, this study found that underweight among preschool children was mainly associated with parental factors. Hence, designing a special nutritional intervention program involving older parents and lower education levels could overcome this problem.

Keywords: children, education level, older parental age, underweight

INTRODUCTION

Malnutrition is defined as deficiencies or excesses in nutrient intake (WHO 2021). It consists of undernutrition, overweight and obesity. Undernutrition is preventable, but it remains a major public health issue as it has been associated with 45% or 3 million deaths of children worldwide (Tan *et al.* 2021; WHO 2021). There are four broad forms of undernutrition–underweight, wasting, stunting and micronutrient deficiencies. Underweight refers to low weight-for-age (WHO 2021). A child's BMI z-score category of <-2SD is classified as underweight (WHO 2019). Childhood underweight has long-lasting implications. Apart from being responsible

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for the highest mortality rate in children, it also linked to poor mental development and school achievement as well as abnormal behaviour. Later in adulthood, it is related to increased risk of several psychiatric disorder and reduced capacity for manual work. Besides the health consequences, undernutrition also affects the economy. Through poor physical condition and learning deficits, there will be loss of productivity. Nonetheless, undernutrition also increases expenses. This will hinder the economic development and prolong poverty (Martins *et al.* 2011).

Among the significant risk factors of underweight are child age, gender, illness, maternal education and household income (Tosheno *et al.* 2017). Children at an early age are more likely to be underweight and the prevalence is higher in males (Khambalia *et al.* 2012). In some studies, children who experience illness had an increased chance of being underweight (Tosheno *et al.* 2017; Menalu *et al.* 2021). However, a child of a mother who had a diploma or higher education level is less likely to be underweight. A significantly lower prevalence of underweight children was also reported in families with higher monthly per capita income (Tosheno *et al.* 2017).

During the last two decades, childhood underweight problems in the less developed Asian countries have been increasing (Mak & Tan 2012). In Malaysia, the prevalence of underweight among children under 5 years old was 13.7% (NHMS 2016) and it persisted in the poor rural areas (Baharudin *et al.* 2019). As evidence-based-practice leads to quality intervention, this study aims to determine the prevalence and investigate the determinants of underweight in preschool children to offer additional evidence for future intervention planning.

METHODS

Design, location, and time

Malaysia is a Southeast Asian country. It includes Peninsular Malaysia or West Malaysia and East Malaysia which is located on the island of Borneo. There are 13 states and a federal territory which covers an area of 333,000 square kilometres (Bowden *et al.* 2013). The east coast region or the peninsular's cultural heartland consists of three states including Terengganu. Terengganu was selected for this study primarily because of lack of data from this area. The preschools were set up by the Department of Community Development (KEMAS) of the Ministry of Rural Development. These public preschools provide early childhood education for children aged 4 to 6 years. Funded by the Ministry of Rural Development, the preschools followed the National Preschool Curriculum since 2003 (Abu Bakar 2016).

This cross-sectional study was conducted from April to September 2019. There were 28 preschools involved in this study. Each was managed by a teacher and an assistant. They were contacted to set the date and time available for every meeting. During the first meeting, they were explained further about this study and given sets of questionnaires to be taken home by the children and filled in by their parents. In this study, parents refer to either father or mother of preschool children. During the second meeting, all children and parent participants gathered and completed any incomplete section in the questionnaires. Their anthropometry measurements were taken by trained enumerators. This study was approved by The Human Research Ethics Committee. The permission to carry out this study at the preschools was obtained from the Ministry of Rural Development Malaysia (KEMAS.BPAK 620-02/01/01 Jld 6 (28).

Sampling

Terengganu is divided into 8 districts. By using the lottery method of simple random sampling, three districts – Besut, Kemaman and Kuala Nerus were chosen as study locations. A list of public preschools was obtained and 10 preschools which situated in each district were chosen randomly. Out of 30, only 28 preschools were included. The remaining 2 were excluded due to permanent closure and participation reluctance. Preschool children participants' recruitment was based on having a parental participation. We included preschool children aged 3 to younger than 7 years old. Preschool children or parents with physical and mental disabilities and pregnant mothers were excluded. The sample size (n) was calculated using the single proportion formula for prevalence (Naing *et al.* 2006; Arifin 2013).

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

For α=0.05, z=1.96 for a two-tailed test. The expected prevalence (p) was 13.7% and the precision (d) was 0.05. By including dropout of 20%, a minimum of 218 participants were included.

Of all 350 questionnaires distributed, 220 were returned. But 2 of them were incomplete, resulting in a response of only 218 preschool children. They were 104 males and 114 females aged 39 to 77 months old whilst their parents or legal guardians were 28 males and 190 females aged 21 to 63 years old.

Data collection

Sociodemographic data were collected using a set of self-reported questionnaires which was developed based on several validated questionnaire from previous studies (Katzmarzyk *et al.* 2013; Norimah *et al.* 2014). The questionnaire consisted of 10 sections. It was available in both Malay and English languages.

All participants' height and weight were taken and recorded by trained enumerators in duplicate to ensure accuracy. Their height was measured to the nearest 0.1 cm using a Seca 213 portable stadiometer without shoes (Seca, Germany) whilst weight was measured to the nearest 0.1 kg using a calibrated Tanita body composition analyzer (TANITA, Japan). The children BMI for-age z-score was calculated using the WHO AnthroPlus software, Version 1.0.4 anthropometric calculator. Data such as the date of measurement, participant's gender, date of birth, participant's weight and height were entered to calculate BMI. Categories of BMI z-scores for children were defined as follows: underweight, ≤2SD; normal, -2SD to +1SD; overweight, >1SD to +2SD; and obese, ≥2SD (WHO 2019). The parents' BMI was obtained from the body composition analyser. Parents' BMI results were categorized as follows: underweight, <18.5; normal, 18.5–24.9; overweight, 25–29.9; obese class I, 30–34.9; obese class II, 35–39.9; and obese class III, ≥40.

Data analysis

All data were analysed using IBM SPSS Statistics for Windows software, Version 22.0 (IBM Corporation, Armonk, New York, USA) licensed to Universiti Sains Malaysia. Descriptive statistics were used to describe most of the participants' characteristics in means and their standard deviation or prevalence percentage. The normal distribution was tested by using the Kolmogorov-Smirnov. An independent t-test was used to test the difference of mean of the variables. For logistic regression analysis, underweight children were coded as 1; and normal, overweight or obese children were coded as 0. One-way Univariate Analysis of Variance (ANOVA) was used to assess the relationship between age of parents and level of education. In binary logistic regression analysis, each determinant factor having a $p<0.25$ was considered for multivariable analysis. Adjusted odds ratios (aOR) with a 95% Confidence Interval (CI) were calculated for predictor variables with $p\leq0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Table 1 shows sociodemographic characteristics of 218 preschool children with a mean age of 5±1 years old, ranging from 3 to younger than 7 years old. More than half of the children were female (52.3%) and had 3±1 number of siblings. Most children had normal birth weight (89.9%) and were delivered full term or post term (87.6%). The mean duration of breastfeeding and formula feeding were 16±11 and 20±19 months respectively. The duration of breastfeeding in this study was comparable with previous study (15.0±7.6 months) (Noraida *et al.* 2017). Our findings show that children are introduced to eat solid foods at the age of 6 ± 2 months, which is acceptable. There is growing evidence of the importance of complementary feeding that may affect both short- and long-term effects on optimal growth, body composition, neurodevelopment, and healthy food preferences (Campoy *et al.* 2018).

Table 2 represents the sociodemographic data of parents with mean age of 35±7 years old. Most of them had secondary school education both among fathers (63.0%) and mothers (57.6%). Most fathers were working (92.6%) whilst majority of mothers were not working (66.0%). The mean number of dependent and household members was 4±1 and 5±1 respectively. Most parents reported a monthly income of less than RM4,851 (USD1,158), (92.7%). It was reported that majority of fathers had a normal BMI (51.9%). In contrast, mothers were more prevalence (53.9%) of being overweight or obese.

Overall, the prevalence of underweight, normal, overweight and obese among the children were 17.9%, 73.8%, 4.6% and 3.7% respectively

Table 1. Sociodemographic distribution of preschool children in Terengganu

Variable	n=218 (%)	Mean±SD
Gender of preschool children		
Male	104 (47.7)	
Female	114 (52.3)	
Age, years		5±1 (3−6 years)
<4	19 (8.7)	
4−<5	89 (40.8)	
5−<6	77 (35.3)	
6−<7	33 (15.1)	
Number of siblings		3±1
<2	84 (38.5)	
≥2	134 (61.5)	
Birth weight		
Normal	196 (89.9)	
LBW/VLBW/ELBW	22 (10.1)	
Birth category		
Post term/full term	191 (87.6)	
Moderate to late term/ very preterm/ extremely preterm	27 (12.4)	
Duration of breastfeeding, months		16±11
Duration of formula feeding, months		20±19
Age starts solid food, months		6±2

LBW: Low Birth Weight; VLBW: Very Low Birth Weight; ELBW: Extremely Low Birth Weight

(Table 3). Our finding on underweight prevalence was much higher compared to the national prevalence which was 14% (NHMS 2019). The discrepancies may be due to the national data including the general population while our study focuses only on preschool children on the east coast of Peninsular Malaysia. Lee *et al.* (2021) also reported a lower prevalence of underweight among preschool children as compared to this study. The lower prevalence was due to previous study involve preschool children living in the urban capital city of Malaysia. However, children from rural area located at Tuba Island reported a slightly higher prevalence of underweight which was 22.4% (Salleh *et al.* 2021). The skewed improvement in certain places while other places remained stagnant might be attributable to the range of strategies and implementations done by the government. Though the prevalence of underweight has decreased over the past decades, close monitoring is needed to ensure Malaysian children are able to grow healthily. This study was in line with previous study by Lee *et al.* (2021) which showed no difference in BMI classification of male and female preschool children.

In multivariate logistic regression, variables with p<0.25 in binary logistic regression such as age of preschool children, birth weight, age of parents, education level and BMI of parents were included into the model (Table 4). In the final model, only the age of parents and education level were found to be significant determinants of underweight. However, there was no significant relationship between age of parents and education level (one-way ANOVA, F=2.054, p=0.131). As the age of parents increases, there is a 1.065 times higher risk of preschool children to be underweight (p=0.033 ; OR=1.065; 95% CI:1.005−1.129). Previous study has highlighted the role of caregiver or parent as one of the most relevant explanatory variables of children's health issues (Khattak *et al.* 2017). As parents age, the children have more risk of being underweight. This scenario may be due to increased commitment and burden among caregivers, resulting in less time spent to feed children. Previous research has found that levels of housework, combined with having more children and increased parenting responsibilities from work, often contribute to women's physical discomfort (Owoo & Lambon-Quayefio 2021). Lack of food quality, poor child feeding, and care practices are often thought to be the primary causes of child malnutrition (Chowdhury *et al.* 2016). It was found that the parenting practices will determine the extent, quality, quantity and manner of children on food (Vollmer & Baietto 2017). Parental responsibility is one of the main reasons for insufficient duration of breastfeeding or complementary feeding. Parents need to take the time to learn parenting practices related to responsiveness to the child's needs (Vollmer 2019).

In addition, parents with a lower level of education were reported to have a 4.383 times

Table 2. Sociodemographic distribution of parents in Terengganu (n=218)

Variable	Father		Mothers		Parents	
	n (%)	Mean±SD	n (%)	Mean±SD	n (%)	Mean±SD
Age, years		36±8		35±6		35±7
Education level						
Primary	1 (3.7)		10 (5.2)		11 (5.0)	
Secondary	17 (63.0)		110 (57.6)		127 (58.3)	
Tertiary	9 (33.3)		71 (37.2)		80 (36.7)	
Employment status						
Working	25 (92.6)		65 (34.0)		90 (41.3)	
Not working	2 (7.4)		126 (66		128 (58.7)	
Number of dependents					4±1	
Number of household member					5±1	
Household monthly income						
B40 (<RM4,851)	25 (92.6)	25 (92.6)		177 (92.7)		202 (92.7)
M40 (RM4,851−RM10,970)	2 (7.4)	2 (7.4)		14 (7.3)		16 (7.3)
Body mass index classification						
Underweight	1 (3.7)	1 (3.7)		13 (6.8)		14 (6.4)
Normal	14 (51.9)	14 (51.9)		75 (39.3)		89 (40.8)
Overweight/Obese	12 (44.4)	12 (44.4)		103 (53.9)		115 (52.8)

B40: Refers to the lowest 40% household income group with average monthly income below RM4,851 (USD:1,158); M40: Refers to the middle-income household group with average monthly income ranging from RM4,851 to RM10,970 (USD:1,158−2,619) according to Department of Statistics Malaysia (2020)

Table 3. Association between body mass index for age (BMI-for-age) and gender among preschool children

BMI-for-age	Male	Female	Total	Fisher's exact test	p
Underweight	20 (19.2)	19 (16.7)	39 (17.9)	2.942	0.401
Normal	74 (71.2)	87 (76.3)	161 (73.9)		
Overweight	4 (3.8)	6 (5.3)	10 (4.6)		
Obese	6 (5.8)	2 (1.8)	8 (3.7)		

No significant different at p>0.05 using fisher's exact test

higher risk for their children to be underweight (p=0.042; OR=4.383; 95% CI:1.054−18.232). This finding suggests the education level of parents is strong determinant of underweight. This is in line with previous study which found that being born into families/parents with low education level were associated with poor nutrition (wasting and underweight) compared to children born from university or college-educated families (Menalu *et al.* 2021). Parental education level is one of the important factors for child development. Poor education levels increase vulnerability to food insecurity and can affect children's feeding (Abdul Talib *et al.* 2020). A better nutritional status leads to a lesser chance of having developmental delay (Huiracocha-Tutiven *et al.* 2019). Guidance for parents on balancing nutrition helps in engaging their children to achieve a healthy weight.

Despite the important findings to fill the knowledge gap about the phenomena in Terengganu, there are several limitations of this

Table 4. Determinants of underweight among preschool children and their parents

Determinant variables	Crude p	Adjusted p	AOR	95% CI Lower limit	95% CI Upper limit
Gender of preschool children					
Male	0.622	-	-	-	-
Female					
Age, years					
<4	0.138	0.504	1.752	0.338	9.085
4−<5		0.190	2.262	0.669	7.659
5−<6		0.870	1.114	0.305	4.062
6−<7		Ref	Ref	Ref	Ref
Number of siblings	0.971	-	-	-	-
Birth weight					
Normal	0.241	Ref	Ref	Ref	Ref
LBW/VLBW/ELBW		0.085	2.567	0.879	7.492
Birth category					
Post term/Full term	0.254	-	-	-	-
Moderate to late term/very preterm/extremely preterm					
Duration of breastfeeding	0.716	-	-	-	-
Duration of formula feeding	0.535	-	-	-	-
Age starts solid food	0.351	-	-	-	-
Age of parents	0.089	0.033*	1.065	1.005	1.129
Education level of parents					
Primary	0.175	0.042*	4.384	1.054	18.232
Secondary		0.851	0.925	0.411	2.421
Tertiary		Ref	Ref	Ref	Ref
Employment status of parents					
Working	0.300	-	-	-	-
Not working					
Number of dependents	0.400	-	-	-	-
Number of household member	0.304	-	-	-	-
Household monthly income					
B40 (<RM4,851)	0.444	-	-	-	-
M40 (RM4,851−10,970)					
Body mass index classification of parents					
Underweight	0.244	0.844	1.162	0.261	5.183
Normal		0.904	1.053	0.458	2.421
Overweight/Obese		Ref	Ref	Ref	Ref

CI: Confidence Interval; AOR: Adjusted Odds Ratio; Ref: Reference Group; LBW: Low Birth Weight; VLBW: Very Low Birth Weight; ELBW: Extremely Low birth Weight; B40: Refers to the lowest 40% household income group with average monthly income below RM4,851 (USD:1,158); M40: Refers to the middle-income household group with average monthly ranging from RM4,851 to RM10,970 (USD:1,158−2,619) according to Department of Statistics Malaysia (2020); *Significant at $p<0.05$ using multiple logistic regression; Variables with $p<0.25$ in the binary logistic analysis were included to the final model for adjustment (AOR) study. As the sample population is specific, this study should be followed up by a large-scale study that includes all public and private preschools in all districts and states in Malaysia to improve its generalisability. In addition, this study used a cross-sectional design which limits causations. Nonetheless, our findings could provide an overview to alert the authorities for future targeted intervention. A more comprehensive study can be explored to gain a deeper understanding of underweight among preschool children.

CONCLUSION

The higher prevalence of underweight among preschool children in Terengganu (17.9%) is higher than the national prevalence, raising national health concerns. Parental age and education level were identified as the risk factors. As underweight linked to negative outcomes, it is important to raise awareness and implement preventive measures targeting these factors in the population. There is a need to implement a special nutritional intervention program involving parents of older age and lower education levels to overcome this problem There is also a need to track feeding practices to provide basic ideas for effective prevention. The potential and holistic strategy on childcare and child feeding may improve children's health and reduce the risk of underweight later in life.

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DECLARATION OF INTERESTS

The authors have no conflict of interest.

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The Effect of Sports Drink Gel Treatment from Chia Seeds (*Salvia hispanica* L.) on the VO_2 Max Capacity of Football and Futsal Players

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ABSTRACT

The purpose of this study was to determine the effect of sports energy gel drink from chia seeds (*Salvia hispanica* L.) on the VO_2 max capacity of football and futsal players in Semarang. This is a quasi-experimental research using a crossover design on twenty-two (22) football and futsal players residing in Semarang, Indonesia. The duration of the study was five weeks, whereby 22 subjects in the control group were given 300 ml of mineral water added with butterfly pea extract as natural colorant, while another 22 subjects in the treatment group were given 300 ml of sports gel drink from chia seed 30 minutes before starting the exercise, twice a week. Data on weight, height, body fat percentage, energy and nutrient intake, and VO_2 max capacity were collected. The results showed that there was a significant increase in the player's VO_2 max capacity value after treatment with sports gel treatment (p=0.001), but there was no significant increase in the control group (p=0.314). Nutritional status, body fat percentages, physical activity, energy intakes, macro and micronutrients intake were found not to be correlated with VO_2 max capacity. It can be concluded that administration of 300 ml sports gel drink from chia seeds before training increases the player's VO_2 max capacity.

Keywords: chia seeds, football, futsal, sports drink gel, VO_2 max capacity

INTRODUCTION

Football and futsal are types of sports that are favored by many people, from children, teenagers, to adults (Nasution 2018; Aswad & Amir 2015). In line with the development of these two sports, training and coaching efforts are carried out to obtain good achievements at the regional, national and international levels.

Football and futsal have several things in common. This can be seen from the characteristics of both, which both use the field, several players working together to put the ball into the goal guarded by the goalkeeper. However, in futsal, the size of the ball, field and goal is smaller than in football. The game of futsal relies heavily on the high technical ability of all players. The pattern of play in futsal is dominated by foot-to-foot games, both in attacking and defending patterns, which tend to rely on short passes given the smaller field size compared to football fields (Badaru 2017).

One of the main factors that must be owned by futsal and football players is endurance. If the endurance and physical fitness of players is low, this will have an impact on the decline in player performance, especially in speed and skills in playing football (Amin *et al.* 2017). To find out the endurance of football or futsal players, it can be done by measuring the VO_2 max capacity. The high and low VO_2 max capacity of the players greatly affects the physical condition or physical fitness of the players themselves (Busyairi & Ray 2018).

Good nutritional status and balanced nutritional intake can support optimal cardiorespiratory endurance. The results of research conducted by Widiastuti et al showed that from a total of 26 athletes recruited as subjects, 22 athletes (86.4%) consumed energy according to their needs and had a VO_2 max capacity value that met the standards and the remaining four athletes (15.4%) consume less energy than they need to have a VO_2 max capacity less than standard. VO_2 max capacity is the highest amount of oxygen that can be received and utilized by individuals to produce energy (ATP) through aerobic metabolism while breathing air during exercise or strenuous exercise. An individual's cardiorespiratory capacity can be determined by measuring the VO_2 max capacity (Plowman & Smith 2014).

A balanced intake of nutrients affects the appearance of an athlete at the time of competition. Consumption of energy and nutrients that are less or more than the total needs, in general, will have

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an unfavorable effect on the body's physiological functions (Amin *et al.* 2017). Endurance athletes need the same macronutrients as other athletes but in higher amounts to help meet energy needs during training or competition. The main difference in dietary regulation between endurance athletes and other sports athletes is in the regulation of the amount of food consumed. Higher calorie needs in endurance athletes emphasize the availability of the body's energy reserves, especially glycogen stores to be used for a long period of time such as a futsal match that lasts 2x20 minutes in normal time or even a soccer match that lasts for 2x45 minutes of normal time (Fink & Mikesky 2018).

Sports drinks consumed during exercise can help improve exercise performance because they contain important energy and electrolytes that can provide adequate amounts of fluids (Gujar & Gala 2014) Unfortunately, athletes are not able to consume or receive a lot of fluid intake in one drink, especially during exercise. On the other side, athletes really need adequate intake of energy, nutrients and electrolytes to support their performance during exercise. For this reason, athletes need to be given sports drinks that are dense of energy, nutrients and electrolytes, but with not too much fluid. This can be applied through sports drink products in the form of gel (Lestari *et al.* 2020).

The food component that can be used as a source of energy and nutrients in sports drink products is chia seeds (*Salvia hispanica* L.). Chia seeds are small grains that are oval in shape, black, grey, or brown in color, and are accompanied by white spots. In 100 g of chia seeds, there is a total of 486 kcal of energy, 16.54 g of protein, but without gluten (Safari *et al.* 2016). The fat in chia seeds is also high (30–40% of the weight of the seeds, 60% of the total fat is omega three fatty acids) (Safari *et al.* 2016). Chia seeds also contain high dietary fibre as much as 34.4 g (>30% total weight, 5–6% in the form of gum) (Reyes-Caudillo *et al.* 2008). Not only rich in nutrients, Chia seeds also contain polyphenols (such as gallic, caffeic, chlorogenic, cinnamic and ferulic acids, quercetin, kaempferol, epicatechin, rutin, apigenin and p-coumaric acid) and isoflavones (such as daidzein, glycitein, genistein and genistin) which are found in small amounts (Kulczyński *et al.* 2019).

Looking at the amount of nutritional content in chia seeds, further research needs to be done to investigate the efficacy of chia seeds sports gel oral administration on the VO_2 max capacity in football and futsal players.

METHODS

Design, location, and time

This is quasi-experimental research, using a crossover design approach. The study was conducted at Universitas Diponegoro Football field, Pleburan, and Manunggal Jati Sports Building, Semarang, Indonesia. The research was carried out from July to August 2021. The study obtained approval from the Ethics Review Committee of the Public Health Department, Universitas Negeri Semarang (163/KEPK/EC/2021).

Sampling

The sample was selected using a total sampling technique based on several criteria: the gender is male, registered as a member of the T-Rex Jawa's football and futsal club, aged 15–19 years, actively participates in training at least once a week, has no history of the cardiorespiratory disease (Upper respiratory infection, Asthma, Tuberculosis, Congestive Heart Failure and Chronic obstructive pulmonary disease), non-smoker, not injured, not taking supplements regularly, and agreeing to participate after signing the informed consent and get approval from parents and coach. Subjects are excluded from the study if sick and withdrawn from the study. Subjects were recruited using the total sampling method, where the entire population that meets the criteria would be used as the research sample (Sugiyono 2015). The total of populations that meet the criteria are 22 people, therefore the total sample size per group is 22 subjects.

Supplement preparation

The formulation to make sports energy gel in this study was based on modifications from Lestari *et al.* (2020) and Lestari *et al.* (2021) including: 4 g of chia seeds, 5 g of maltodextrin, 0.2% xanthan gum, 300 ml of coconut water, and 0.1% butterfly pea (*Clitoria ternatea*) juice. A sports energy gel is produced by mixing chia seeds, maltodextrin and xanthan gum in to 300 ml of coconut water, all ingredients are homogenized and added butterfly pea juice to give colour, and then heated over low heat for 10 min, until its boils (Lestari *et al.* 2020). Once finished, the sports energy gel is then packaged in a 300 ml plastic pouch.

Placebo is made by mixing 300 ml of boiled water with butterfly pea juice as a colourant to produce the same colour as sports energy gel from chia seeds. Then packed in a 300 ml plastic pouch.

Data collection

Anthropometric and body composition. Anthropometric measurements and body composition of subjects included weight, height, and body fat percentage. Body weight and body fat percentage were measured using Bioelectric Impedance Analysis (BIA), and height was measured using a statue meter (0.1 cm precision level).

Determination of nutritional status. Determination of nutritional status using BMI for age anthropometric index because the subject is adolescents aged 14–18 years. Body mass index according to age is calculated based on the results of measurements of weight and height which is then associated with age parameters. The measurement results were then categorized into underweight, normal weight, and overweight (MoH RI 2020). In addition to BMI according to age, percent body fat was also used as an indicator for assessing the nutritional status of the subject. The results of the percent body fat measurement were then categorized into skinny, good, and acceptable body fat (Heyward & Wagner 2004).

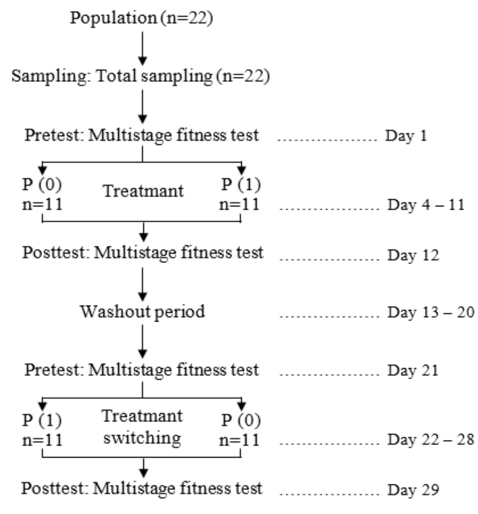
Assessment of food intake. Food intake was obtained from interviews with subjects using multiple recall 24 hours (non-consecutive days) after treatment and cardiorespiratory endurance tests. The results of the interview were then converted into calories and grams of macronutrients and then compared with the Recommended Dietary Allowance (RDA) (MoH RI 2019) for adolescents aged 14–18 years multiplied by 100%.

The level of energy adequacy and nutrients is then categorized into three groups, namely deficit (<80% RDA), adequate (80–110% RDA) and excessive (>110% RDA) for energy, carbohydrate, fat and protein intake (Widyakarya Nasional Pangan dan Gizi 2012). And for mineral intake, it was categorized into two groups, namely deficit (<77% RDA) and adequate (≥77% RDA) (Gibson 2005).

Assessment of energy expenditure. The energy expenditure assessment was carried out by interviewing the subject's physical activity using the long form IPAQ to determine the subject's average daily METs (metabolic equivalent of task). The results were then classified into low, normal, and hard (IPAQ Research Committee 2005; WHO 2012).

Treatment and cardiorespiratory endurance test. On the first day, all subjects performed a cardiorespiratory endurance test using the Multistage Fitness Test (MFT) without any prior treatment for the pretest data. After that, the subjects will be divided into two groups, into control [p(0)] and experimental [p(1)]. The control group [p(0)] received 300 ml placebo and the experimental group [p(1)] received 300 ml sports energy gel. Treatment was given twice in 1 week following the subject's training schedule. After the treatment, subjects performed a cardiorespiratory endurance test for posttest data. After that, the subject was freed from any treatment and measurement for one week, the aim was to rest the subject and eliminate the effects of the previous treatment (washout period) (Figure 1).

After completing the washout period, all subjects performed a cardiorespiratory endurance test again for pretest data. then, the subjects were again divided into the same two groups as before, but now the control group received 300 ml of sports energy gel [p(0)→p(1)] and the experimental group received 300 ml of placebo [p(1)→p(0)]. Treatment was given twice in 1 week following



P(0): Control group; P(1): Experiment group

Figure 1. The stages of treatment and cardiorespiratory endurance test

the subject's training schedule. Then, the subjects performed a cardiorespiratory endurance test again for posttest data. Therefore, the total number of subjects in the control and treatment groups was 22 subjects [p(0)=22 and p(1)=22].

Cardiorespiratory endurance test

The cardiorespiratory endurance test with 20-meter shuttle run (multistage fitness test), is appropriate for measuring cardiorespiratory endurance in youth (Pate *et al.* 2012). The shuttle run is advantageous when there are time constraints and when cost may be a problem, such as in schools and other educational settings (Pate *et al.* 2012). In a review article, Artero *et al.* (2011) report that test-retest reliability coefficients for this test have ranged from r=0.78 to r=0.93. Overall, the available evidence suggests that the 20-meter shuttle run has excellent validity and reliability as a measure of cardiorespiratory endurance. Procedure of multistage fitness test: a) The subject runs in a 20 meters track and if the subject arrives at the end of each lap before the "beep" sound, then the subject must wait for the "beep" to then continue running on the next lap; b) The subject must keep his running speed in accordance with the rhythm of the "beep" sound and if the subject is no longer able to follow 3 "beeps" in a row, the multistage test ends for that subject; c) The value of the multistage test results is then converted into a table for calculating VO_2 max capacity predictions.

Data analysis

Univariate analysis was used to describe all variables in the study. Bivariate analysis was used to determine the differences of VO_2 max capacity before and after the intervention in both the treatment and control groups. The bivariate analysis begins with testing the normality of the data. The normality test on each variable used the Shapiro-Wilk test because the total number of samples was less than 50 people. Results of the normality test showed that the data were not normally distributed, the next test selected was the Wilcoxon test (non-parametric test) and the Mann Whitney test (Amin *et al.* 2017; Dahlan 2014). The results of the analysis are said to be significant if the probability value is less than 5% (p<0.05) (Dahlan 2014).

RESULTS AND DISCUSSION

The total subjects in this study were 22 young male athletes. Some subjects are still in high school and some have completed high school education with an age range of 15–19 years. The subject's height range was 149.4–185.2 cm and the subject's weight range were 38.1–82.6 kg. The complete characteristics of the research subjects are presented in Table 1.

The nutritional status of the subjects was based on BMI for age; 18 subjects had normal nutritional status, one subject was underweight, and three subjects were overweight. The percentage of body fat of the subject is between 7.40–24.10 %; nine subjects were classified as skinny, eight subjects were classified as good, and five subjects were classified as acceptable. The subject's energy expenditure ranged from 674 to 1,781 kcal per day with details of 18 subjects classified as normal, and four subjects were classified as hard. The complete description of energy and nutrient intake of research subjects is presented in Table 2.

The results of the analysis showed that most of the energy and macronutrient intakes of the subjects were classified as deficits. and the

Table 1. Characteristics of subjects in control and treatment groups

Variables	Control (n=22)		Treatment (n=22)		p*
	Mean±SD	Median	Mean±SD	Median	
Age (years)	17.1±1.2	17.0	17.1±1.1	17.0	0.941
Height (cm)	166.1±8.9	166.2	166.2±8.9	166.3	0.907
Weight (kg)	58.2±10.5	58.5	58.3±10.5	58.5	0.897
BMI for age (z-score)	-0.30±1.1	-0.62	-0.29±1.1	-0.62	0.916
Fat mass (%)	14.94±4.6	14.90	14.92±4.5	14.80	0.888
Energy expenditure (kcal)	950±392.7	847	946±359.3	847	0.860

*Non-parametric test: Mann-Whitney test; Significantly different at p<0.05; BMI: Body Mass Index

Table 2. Average energy and nutrient intakes in control and treatment groups

Food Intake	Control (n=22)		Treatment (n=22)		p*
	Mean±SD	Median	Mean±SD	Median	
Energy (kcal)	1309.83±237.54	1203.00	1281.4±185.28	1189.30	0.814
Protein (g)	48.29±14.03	45.95	47.66±12.04	46.55	0.888
Fat (g)	38.44±17.16	38.70	34.75±14.24	29.20	0.526
Carbohydrate (g)	188.87±41.72	190.55	190.66±33.64	192.05	0.991
Sodium (mg)	342.49±314.98	182.40	171.80±127.74	162.50	0.178
Calcium (mg)	158.51±78.26	134.00	132.53±35.76	131.65	0.439
Potassium (mg)	897.42±350.88	868.70	837.90±259.84	820.55	0.589
Magnesium (mg)	163.23±46.73	162.85	219.32±309.73	156.55	0.851

*Non-parametric test: Mann-Whitney test; Significantly different at $p<0.05$

mineral intake of sodium, calcium, potassium and magnesium are mostly also classified as deficit. The results of the bivariate analysis to determine the difference in the subject's VO_2 max capacity value are presented in Table 3.

The end line results showed that the group who received the treatment of 300 ml chia seed drink had a higher average in VO_2 max capacity value (42.17±6.32 ml/kg/minute) than the control group receiving 300 ml placebo (38.11±6.37 ml/kg/minute). There was an increase in VO2 max capacity value of 1.97 ml/kg/minute in subjects who received the sports energy drink from chia seeds. It was equal to five times the rate at the 8th level on the Multistage Test (about 30 seconds). This value is very meaningful when applied in football and futsal matches, for example when all players with the same cardiorespiratory endurance ability are already in a state of ability threshold (fatigue), one of the players has a VO_2 max capacity greater than 1.97 ml/kg/minute, then the player still has the additional ability to run for another 30 seconds.

The results of this study are in line with the research of Illian *et al.* (2011) which showed that a mixture of chia seeds and a 6% carbohydrate sports drink supported a 10 km running performance (after running at a moderate intensity for one hour) to the same level as an isocaloric volume of the 6% carbohydrate sports beverage alone. Another study also found that ingesting 7 kcal kg-1 of chia seed oil 30 minutes before running at 70% VO_2 max capacity led to a 3.4-fold increase in plasma Alpha-Linolenic Acid (ALA) levels (Nieman *et al.* 2015). The addition of chia seeds (Figure 2) as a component in sports drinks can increase energy content while lowering the amount of fluid in sports drinks, as athletes are expected to consume high-calorie drinks with few fluids (Lestari *et al.* 2021). Athletes who perform high-intensity endurance exercises are restricted from consuming excessive fluids during exercise, since they can cause discomfort to the digestive system (Shirreffs 2009; Lestari *et al.* 2020).

The nutritional status of subjects based on BMI for age is not related to VO_2 max capacity ($p=0.669$). The results are similar to the research conducted by Widyastari and Setiowati (2015) which showed that there was no significant effect between nutritional status and VO_2 max capacity. Research conducted by Amin *et al.* (2017) also showed the same results that there was no significant relationship between nutritional status and VO_2 max capacity of soccer athletes. Body fat percentage is also an important factor that can support the physical condition and performance of the players in addition to nutritional status. The higher the proportion of body fat and the

Source: Vecteezy (2018)

Figure 2. Chia seed (*Salvia hispanica* L.)

Table 3. VO_2 max capacity values in control and treatment groups

Groups	Control		Treatment		p^*
	Mean±SD	Median	Mean±SD	Median	
Pretest	37.69±7.26	37.01	40.20±6.46	38.48	0.038^*
Posttest	38.11±6.37	35.84	42.17±6.32	40.87	
Δ VO_2 max capacity	0.41±2.79	1.29	1.97±2.07	2.72	0.023^{**}

*Non-parametric test: Wilcoxon test; **Non-parametric test: Mann-Whitney test; Significantly different at $p<0.05$

increase in the endomorphy of the body shape of a player or athlete, the acceleration of a person's movement will decrease. Physical movement in sports is closely related to skills related to fitness or fitness related to skills (Saputra *et al.* 2019). The results of statistical analysis shows that the proportion of body fat is not significantly related to the subject's VO_2 max capacity (p=0.528). This is similar to the results of research conducted by Amin *et al.* (2017) which showed that there was no significant effect between body fat percentage and VO_2 max capacity of soccer athletes (p=0.43). However, this result is different from the research of Dewi *et al.* 2015 which explains that the proportion of body fat is significantly correlated with VO_2 max capacity (p<0.001).

Apart from nutritional status (BMI for age) and body fat percentage, this study also analyzed the subject's physical activity factors. The description of the subject's physical activity during the study was mostly classified as normal or moderate, namely 18 subjects and the activities of four other subjects were classified as heavy. The results of statistical analysis shows that physical activity has no significant effect on the subject's VO_2 max capacity (p=0.586).

Factor analysis of the subject's food intake showed that there was no significant effect between energy, protein, fat and carbohydrate intake with VO_2 max capacity of subjects with p-values of energy 0.356, protein 0.132, fat 0.942 and carbohydrates 0.597 (p>0.05). This result is in line with research Safitri & Dieny (2015); Setiawan (2016); Amin *et al.* (2017) which showed that the intake of energy, protein, fat and carbohydrates did not have a significant effect on the VO_2 max capacity of football athletes. In addition to energy and nutrients such as protein, fat and carbohydrates, the intake of minerals such as magnesium, calcium, sodium and potassium is also a confounding variable in this study. The magnesium intake of subjects in the control group, there were 16 subjects included in the deficit category, five subjects classified as adequate, and one subject classified as excessive, and in the treatment group there were 18 subjects included in the deficit category, three subjects classified as adequate, and one subject classified as excess. Likewise with sodium, calcium, and potassium intake, where all subjects in both the control and treatment groups were included in the deficit category.

In addition to energy intake and macronutrients, this study also analyzed the subject's mineral intake during the study. Based on the results of this study, it is known that there is no significant effect between intake of sodium, calcium, potassium, and magnesium, with the subject's VO_2 max capacity with p-values respectively 0.396 sodium, 0.780 calcium, 0.126 potassium, and 0.141 magnesium (p>0.05). This is similar to the research conducted by Amin *et al.* (2017) which showed that the intake of sodium, calcium, potassium and magnesium did not have a significant effect on the VO_2 max capacity of football athletes.

This study showed that increase in physical endurance is mostly resulted from the increase in energy intake from the chia seed drink, thus did not reflect balanced energy and nutrients intake as main requirements for determining the level of work productivity (MoH RI 2011). The portrayal of imbalance nutrient intake in this study can be caused by the 24-hours food recall results during the VO_2 max capacity test were not consecutive thus insufficient to meet their needs. In addition, the body's ability to use oxygen optimally can be determined by factors other than food intake (Setiawan 2016).

Treatment is not given for one full week, but is only given two times a week following the subject's training schedule due to the Covid-19 pandemic. And analysis of food intake only for energy, macronutrients and several types of minerals without analyzing vitamin intake became limitations in this study. Recommendations for future research are expected to provide treatment not only two times a week and for a longer time span, so that the effects of sports energy gel on athlete performance can be more optimal.

CONCLUSION

Provision of 300 ml of sports energy drink from chia seed to futsal and football players before exercise can increase the VO_2 max capacity value by 1.97±2.07 ml/kg/minute. This increase in the value of VO_2 max capacity in the treatment group (1.97±2.07 ml/kg/min) was significantly higher than the increase in the control group (0.41±2.79 ml/kg/min) ($p<0.05$). It can be concluded that consumption of sports energy drinks from chia seeds before exercise for endurance athletes such as futsal and football players can provide additional energy needed during exercise.

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DECLARATION OF INTERESTS

The authors have no conflict of interest.

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The Efficacy of Nutrition Education on Anemia and Upper Arm Circumference among Pregnant Women in Aceh Besar District of Indonesia during the Covid-19 Pandemic

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ABSTRACT

This study aimed to analyze the effects of nutrition education on anemia and upper arm circumference in pregnant women. It was a cluster-randomized control study involving 110 pregnant women. The nutrition education interventions, was a combination of offline and online sessions, conducted by trained Nutrition education staffs with a 1:5 ratio to pregnant women. Twelve education sessions were conducted for three months utilizing a nutrition booklet for pregnant women, food monitoring cards, and flyers shared on a social media WhatsApp group. The socio-demographic data were obtained through an interview method. The nutritional status collected, by measuring Mid Upper Arm Circumference (MUAC), and anemia was determined through a diagnosis by the family doctor. Data were analyzed using the chi-square test and the paired and independent t-tests, the confidence interval was set at 95%. Hemoglobin levels in the intervention group experienced a higher increase than in the control group. The MUAC in the intervention group increased by 0.8 cm while in the control group it was decreased by -2.7 cm. However, the Difference in Difference (DID) analysis did not show significant different for both parameters (p=0.198 and p=0.274). Chi square analysis showed that the prevalence of anemia at the end line point in the intervention group (3.6%) was significantly lower compared to the control group (14.5%) (p=0.047). The prevalence of the Chronic Energy Deficiency (CED) measured by MUAC decreased by 9% in the intervention group, and 1.8% in the control group. However, the difference in prevalence of CED was not statistically significant (p=0.696). The nutrition education within three months did not significantly increase the mean hemoglobin and MUAC. But considering the trend in decreasing anemia and CED prevalence in the intervention group, structured and routine nutrition education can be implemented as part of nutritional intervention in pregnant women to prevent anemia and CED to observe effects in a longer-term intervention.

Keywords: anemia, covid-19 pandemic, food monitoring card, nutrition education, pregnant woman

INTRODUCTION

The Covid-19 pandemic has disrupted various sectors, including health care. In Indonesia, SEMERU study showed that the Covid-19 pandemic has generally reduced the number of visits to nutrition, Maternal, and Child Health (MCH) services, changed the implementation of nutrition and MCH services, and influenced access to technology and the internet supports to monitor and consult about nutrition and MCH services (Saputri *et al.* 2020).

The Indonesia Basic Health Research Survey in 2018 reported that Chronic Energy Deficiency (CED) and anemia in pregnant women remain a big nutrition problem in Indonesia. One in three pregnant women suffers from anemia and the trend has been increasing overtime from

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24.5% to 37.10% and 48.9% in 2018 (MoH RI 2019). Study by Tanziha *et al.* also showed similar figure where 38.1% pregnant women in Indonesia suffer from anemia, 38.2% in urban and 37.9% in rural area (Tanziha *et al.* 2016). In addition to anemia, 17.3% of pregnant women in Indonesia suffer from CED (MoH RI 2019).

Malnutrition, including in pregnant women is associated with poor access to nutritious diets, poor access to essential nutrition services, feeding, and dietary practices (UNICEF 2020). The poor nutrition practices started before and continues throughout pregnancy, this includes insufficient intake of energy, nutritional consumption, and micronutrient intakes, such as iron, zinc, calcium, and folic acid (Mousa *et al.* 2019). The compliance of pregnant women in consuming >90 iron and folic acid tablets during pregnancy is low; the number were only 33.3% and 38.1% in 2013 and 2018 respectively (MoH RI 2019). Another risk factor is protein intake, 49.6% of pregnant women in urban areas and 55.7% of pregnant women in rural areas fulfilled only had 80% of the Recommended Dietary Allowance (RDA) for protein (MoH RI 2019).

Ahmad *et al.* (2020b) found that 14.3% pregnant women in Aceh Besar district suffer from malnutrition including wasting or severe wasting. Moreover, the CED prevalence was 12.7%; more than a third (35.7%) of pregnant women suffered from anemia (Hb 11 g/dl), and nearly a third (29.1%) never took iron tablets. Nutritional practices in pregnant women were not optimal because more than a third of pregnant women never consumed multivitamin supplementation during the pregnancy, and more than a third of pregnant women (39.2%) did not use iodized salt.

Pregnant women, in general, still require appropriate nutrition, thorough and detailed information about nutritional practices, and a balanced diet throughout pregnancy. During the Covid-19 pandemic, communications strategies for maternal nutrition programs focuses on consuming healthy and hygienic food and identifying innovative channels to support culturally appropriate messaging on a healthy meal, hygiene, and physical activities, such as social media, television, radio, digital platforms/ mobile phones, and woman needs (WFP/ UNICEF/Global Nutrition Cluster 2020). The joint nutrition statement for maternal, infant, and young child nutrition during the Covid-19 pandemic in Asia and Pacific regions aims to communicate accurate information on maternal, infant, and young child nutrition (FAO/UNICEF/ WFP/WHO *et al.* 2020).

Adapting to the Covid-19 pandemic, nutrition education requires combination of offline and online nutrition education method. Social media interventions was found to be positively associated with increasing physical activity levels, making healthy food choices, and affecting body composition or weight (Goodyear *et al.* 2021). Our preliminary study has developed a nutrition pocketbook for pregnant women as a medium for nutrition education for mothers, cadres, and health workers, and a food monitoring card for a pregnant woman (FMC-PW) that functions as a self-assessment tool to measure nutritional practices (Ahmad *et al.* 2019). The current study will further develop educational media for online platform, such as flyers. This online media will then be delivered through a combination of limited direct education and undirect education via telephone and WhatsApp. It is expected that the developed nutrition education, using the mix of offline and online strategies would increase adherence to iron and folic acid tablet and nutrients intake from daily food which will improve their nutritional status, especially anemia and CED. Therefore, this study aimed to analyze the effects of nutrition education on anemia and upper arm circumference in pregnant women.

METHODS

Design, location, and time

This study aimed to examine the effects of the nutritional education model on nutritional status and anemia in pregnant women, using a cluster randomized control trial design. The sampling method consisting of two stages: choosing a village as a cluster with criteria for the CED prevalence in pregnant women >10% and randomizing the cluster to be assigned as an intervention or control group. This study was conducted from October 2020 to March 2021 at Darul Imarah Public Health Center in Aceh Besar District. The location was chosen considering that pregnant women in this location had a high prevalence of CED and anemia. The preliminary study in the ares found that 12.7% of pregnant women had CED, 14.3% were undernourished before pregnancy, and 35.7 % had anemia.

Sampling

The research subjects were pregnant women in the first trimester at Darul Imarah Health Center Aceh Besar District. The inclusion criteria for the subject were pregnant women in the first and second trimesters with normal pregnancies. Meanwhile, the exclusion criterion was pregnant women with pregnancy complications based on the medical doctor's diagnosis.

The minimum sample size of the study was determined using the assumption of an error rate (α)=5% (Z=1.96) and power of the test (β)=90% (Zβ=1.28). The calculation of sample size employed the following formula (Lemeshow *et al.* 1990). Referring to the research results of Sunuwar *et al.* (2019) in Nepal that the variable of hemoglobin (Hb) levels increased by 0.56±0.40 g/dl in the education group and 0.16±0.82 g/dl in the control group. The minimum sample size was 114. Ethical approval was issued by the Health Research Ethics Commission of Universitas Sumatra Utara No.2251/IX/SP/2020 dated September 14, 2020.

Nutrition education intervention and follow up. The nutrition education in this study combined an offline and online strategies adapted to the Covid-19 pandemic where direct contact between health staffs and research subjects were limited. The nutrition education was followed by monitoring on food consumption (Table 1).

Nutrition education was delivered by combining direct and indirect techniques (offline and online) and utilizing mobile communication devices (cellphones) and WhatsApp. WhatsApp was used to deliver information and nutrition education, the nutrition booklet for pregnant women which has been developed in the previous research stages was used as a reading reference, and

Table 1. Educational model for monitoring food consumption in pregnant women

Nutrition education model	Implementation of nutrition education model
Educational form	Nutrition education is carried out by indirect methods, namely using phone call and social media applications
Key message (key massage)	1. Staple food (three Servings/day): Rice/bread/noodles/potatoes or other staple food 2. Animal side dishes (three servings/day): Fish/egg/shrimp/meat/chicken/duck/other animal side dishes 3. Vegetable side dish (three servings/day): Nuts/tofu/tempe or other preparations 4. Vegetables (three servings/day): Spinach, kale, katuk leaves, tomatoes, long beans, bean sprouts and others 5. Fruits (three Servings/day): Banana, papaya, mango, watermelon, avocado, *sawo*, guava, orange/other 6. Additional meals or snacks: Two times a day, such as; fruit juice one cup/porridge and two slices of cake or biscuit or staple food 100 g rice + one egg 7. Consumption of iron and folic acid supplement: one tablet daily 8. Drink enough water: At least eight glasses a day 9. Clean living behavior: Maintaining personal hygiene and washing hands with soap 10. Actively moving: Routine physical activity every day for at least 30 minutes until sweating/exercise for pregnant women
Message delivery method (delivery platform)	Community-based approach (community-base-platform) with interpersonal/counselling through ofline and online delivery platform
Number of educational sessions	Conducted as many as 12 sessions with a duration of 45 minutes/session for three months
Educational staff (facilitator)	Educational staff are nutritionists with a ratio of 1:5 (one nutritionist assists five pregnant women)
Tools and media	Food Card Monitoring Consumption for pregnant women (FMC-PW) as a tool for nutrition education, maternal nutrition pocket book as a supporting educational media (reading resources for pregnant women and educational staff)
Behavior change theory	Plan Behavior Theory

a food monitoring card (FMC-PW) was used as a self-assessment tool for daily nutrition practices.

The implementation was divided into three forms. First, the distribution of educational media and nutrition practice evaluation tools was carried out directly by trained nutrition education personnel and nutrition staff as field supervisors. In this activity, the team distributed nutrition booklet for pregnant women and FMC-PW and explained how to use them. Direct and face-to-face activities were only conducted at the beginning and the end of intervention activities by implementing health protocols (using masks, maintaining distance, and washing hands with soap or using hand sanitizers before and after direct contact with participants) by both pregnant women and the research team. Second, online nutrition education, namely delivery of key nutritional education messages, was made in flyers and shared on WhatsApp group, through several stages; the researcher sent the flyer to the supervisor then sent it to the educational staff's on the WhatsApp group. Each education staff sent the research target to the WhatsApp group, with a ratio of 1:5. Third, direct phone call in which education personnel provided education about the application of dietary practices based on FMC-PW and information based on the nutrition booklet for pregnant women and provide consultation for problems experienced by mothers.

The eleven nutrition staffs who have been trained before the intervention was supervised by the field supervisors who monitored the implementation of nutrition education in several stages: 1) Input monitoring was conducted on distribution and delivery of education packages in nutrition booklet for pregnant women, FMC-PW, flyers, and key messages via telephone; 2) Process monitoring evaluated pregnant women's compliance with FMC-PW; 3) Output monitoring referred to data collection results (Figure 1).

Data collection

The collected data included socio-demographic characteristics of pregnant women and families using a structured questionnaire, nutritional status with anthropometric measurements of MUAC using standardized measurement procedures (Tang *et al.* 2016) by trained nutritionist. The CED was categorized based on the MUAC <23.5 cm (MoH RI 2020), and anemia status by examining hemoglobin levels using the by Hemocue® by the Family Doctor. Blood sample was drawn using finger-prick capillary blood carried out and interpreted by trained laboratory personnel in the Community Health Center. Anemia is defined as a hemoglobin concentration level below 11.0 g/dl (WHO 2011).

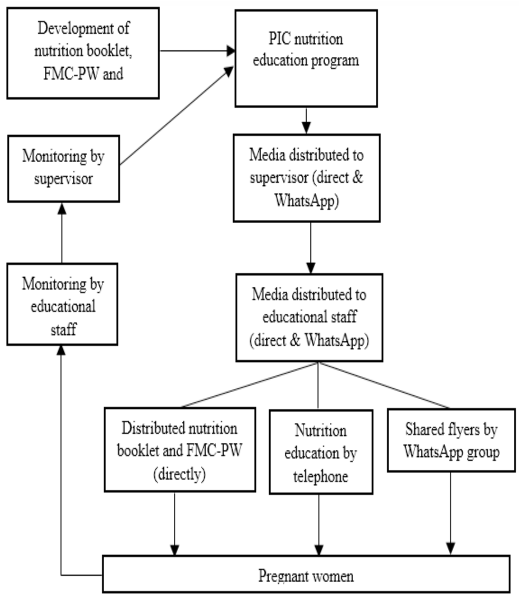
Data analysis

A univariate analysis was used to establish the mean, median, and standard deviation of the MUAC and hemoglobin level, as well as the percentage of anemia prevalence and the CED before and after the intervention. The impacts of the educational intervention were evaluated using an independent t-test for the different mean of the MUAC and hemoglobin and a different in proportion test. To control the confounding variables, the Difference in Difference (DID) test was also conducted. The chi-square test was used to examine the different in prevalence decreases in the CED and anemia. All tests were performed at 95% of confidence level (α=0.05).

RESULTS AND DISCUSSION

Characteristics and socio-demographic family of pregnant women

This study involved 110 pregnant women: 55 of them in the intervention group, and 55 in



FMC-PW: Food monitoring card for pregnant woman; PIC: Person in charge of nutrition education progran

Figure 1. Stages of the nutrition education process

the control group. The study was conducted in the Darul Imarah community health center in Aceh Besar district. The findings (Table 2) showed no significant difference in the socio-demographic characteristics of pregnant women, such as gestational age, education, occupation, family income, number of family members, number of children, and anthropometric status of the mother before pregnancy (p>0.05), only the occupation variable of husbands had significant

Table 2. Characteristics of pregnant women and socio-demographic family

Characteristics of subjects	Intervention group	Control group	p*
Trimester of pregnancy			
First trimester	16 (29.1)	19 (34.5)	0.683
Second trimester	39 (70.9)	36 (65.5)	
Mother's education			
Low (≤SD)	5 (9.1)	8 (14.5)	0.674
Middle/Junior high school	29 (52.7)	27 949.1)	
High (diploma/bachelor)	21 (38.2)	20 (36.4)	
Mother's work			
Housewife	46 (83.6)	45 (81.8)	0.801
Worker	9 (16.4)	10 (18.2)	
Husband's job			
Government	8 (14.5)	4 (7.3)	0.009**
Farmers & fishermen	0 (0.0)	4 (7.3)	
Merchants/entrepreneurs	31 (56.4)	19 (34.5)	
Labour/builder and others	16 (29.1)	28 (50.6)	
Husband's education			
Low (≤elementary school)	2 (3.6)	4 (7.3)	0.662
Middle/junior high school	36 (65.5)	33 (60.0)	
High (diploma/bachelor)	17 (30.9)	18 (32.7)	
Fixed family income (IDR)			
<Rp. 2.7 million	39 (79.1)	38 (69.1)	0.835
≥Rp 2.7 million	16 (29.1)	17 (30.9)	
Number of family members			
4 people	49 (89.1)	46 (83.6)	0.405
5 people	6 (10.9)	9 (16.4)	
Number of children			
Not yet	22 (40.0)	17 (30.9)	0.496
1–2 people	28 (50.9)	30 (54.5)	
3 people	5 (9.1)	8 (14.5)	
Anthropometric status before pregnancy (BMI)			
Wasting	8 (14.5)	6 (10.9)	0.449
Normal	33 (60.0)	29 (52.7)	
Overweight and obesity	14 (25.5)	20 (36.4)	

*: Chi-square test; BMI: Wasting BMI<18.5; Normal: 18.5–27.0; Overweight; BMI>27.0; ** p<0.05; IDR: Indonesian Rupiah

difference ($p<0.05$). This result indicated that the characteristics of the research subjects in the intervention and control groups were relatively similar. Therefore, the influence of the intervention was not expected to be affected.

The effect of nutrition education on pregnant women's anemia and nutritional status

The results (Table 3) indicated no significant difference in the mean of the Hemoglobin (Hb) level between the intervention and control groups in the baseline ($p=0.372$) and after the intervention or end line ($p=0.354$). However, the average increase in Hb levels in the intervention group was slightly higher (0.19 g/dl) than in the control group. After three months of nutrition education intervention, there were no significant effects of the nutrition education on hemoglobin levels (DID: 0.376; 95% CI:0.19–0.95). Similarly, the MUAC in the baseline and in the end line between both groups were also statistically not significant ($p>0.05$) and the MUAC DID analysis was also not significant (DID:1.127; 95% CI:-0.89–3.15). However, in the intervention group's mean MUAC increased by 0.84.00 cm, whereas in the control group it decreased by -2.784.14 cm but the difference was also not statistically significant ($p=0.150$).

However, the analysis of the anemia prevalence (Table 4) before and after the intervention indicated that pregnant women with anemia in the intervention group had decreased by 30.9%, from 34.5% (baseline) to 3.6% (end line). Meanwhile, the control group it decreased by only 16.4%, from 30.9% to 14.5%. In addition, chi square test showed higher chance for anemia in the control groups compared to the intervention group ($p<0.05$).

The findings showed the nutrition education utilizing a combination of offline and online strategies did not significantly increase the average hemoglobin and MUAC level, but significantly reduced the prevalence of anemia. Generally, pregnant women will experience a decrease in hemoglobin with increasing gestational age. The reason is, during pregnancy the blood volume will increase by up to 50 percent to provide important nutrients to the developing fetus. Then starting at eight weeks of gestation, blood plasma levels will be higher than red blood cells in pregnant women. Due to a decrease in the concentration of red blood cells in the blood. Study showed the decrease in hemoglobin during pregnancy is on the order of 1.4 g/dl or 11% of the first trimester level (Churchill *et al.* 2019).

Similar to those of Sunawar et al, our finding also shows that nutrition education interventions and a strict diet in iron sources could increase hemoglobin levels during pregnancy (Sunuwar *et al.* 2019). Another study found individual

Table 3. The effects of nutrition education on the mean of hemoglobin and MUAC in pregnant women before and after the intervention

Haemoglobin and MUAC	Intervention (Mean±SD)	Control (Mean±SD)	p*
Haemoglobin level			
Baseline	11.16±0.90	11.35±1.25	0.372
End line	12.14±0.96	11.95±1.16	0.354
Change	0.97±1.13	0.59±1.53	0.147
DID	0.376 (-0.19–0.95)		0.198
Mid upper arm circumference			
Baseline	26.31±3.41	27.38±3.38	0.100
End line	27.15±4.23	27.10±4.13	0.940
Change	0.8±4.00	-2.78±4.14	0.150
DID	1.127 (-0.89–3.15)		0.274

Independent t-test; Significant $p<0.05$; FMC-PW: Food Monitoring Card Consumption for pregnant women; MUAC: Mid Upper Arm Circumference; DID=Different in different in 95% confidence interval

Table 4. The effects of nutrition education on the anemia and chronic energy deficiency (CED) status in the pregnant mother before and after intervention ntion

Anaemia status and CED status	Intervention group	Control group	p*
Anaemia Status[1]			
Baseline			
Anemia	19 (34.5)	17 (30.9)	0.684
Normal	36 (65.5)	38 (69.1)	
End line			
Anemia	2 (3.6)	8 (14.5)	0.047**
Normal	53 (96.4)	47 (85.5)	
CED status[2]			
Baseline			
CED	8 (14.5)	5 (9.1)	0.376
Normal	47 (85.5)	50(90.9)	
End line			
CED	3 (5.5)	4 (7.3)	0.696
Normal	52 (94.5)	51 (92.7)	

*Chi-square test; [1]: Anemia if Hb<11.0 g/ dl; [2]: Using Mid Upper Arm Circuference (MUAC); CED: Chronic Energy Deficiency; **Statistical significance $p<0.05$

anemia education using a pictorial handbook in conjunction with a counseling intervention program improved hemoglobin and hematocrit levels in anemic pregnant women in their third trimester of pregnancy (Nahrisah 2020). Another study of nutrition intervention education based on the PRECEDE model positively improved iron deficiency anemia (Khani Jeihoon *et al.* 2021) and increased nutritional intake, gain weight, and hemoglobin levels (Soylu 2019).

Nutrition education during the pregnancy promotes maternal and child health, pregnancy outcomes, food intake, micronutrient supplements, and the use of nutrition safety nets (Girard & Olude 2012). Nahrisah *et al.* (2020) examined anemic pregnant women in Indonesia and showed that counseling using handbooks was useful to increase knowledge, maintain adequate nutritional intake, and prevent anemia, as described by the increasing hemoglobin and hematocrit levels.

The results of this study are almost similar to those of Ahmad *et al.* (2020a), researching in Aceh, Indonesia. He discovered that the nutrition education using a structured home visit method coupled with educational media in a pocketbook and an independent evaluation tool in a Food Monitoring Card (FMC) for children aged 6–23 months in undernourished toddlers could improve nutritional status and reduce the prevalence of anemia in undernourished children under five years old (Ahmad *et al.* 2020a).

A study in Southeast Sulawesi showed that nutrition education using Android-based application media improved knowledge, attitudes, and behavior of pregnant women with CED (Lestari *et al.* 2021). Pregnant women's knowledge, attitudes, and practices were improved when the nutrition and reproductive health education administered in small groups using interactive methods (Permatasari *et al.* 2021). Nutrition education is effective if the pregnant women follow the dietary requirements and the food guide pyramid (Khani Jeihoon *et al.* 2021). A systematic review by Chau *et al.* (2018) found that social media was a promising nutrition intervention platform for adolescents and young adults.

Others studies conducted in Purwokerto, Indonesia showed there is an increase in knowledge of nutrition, energy intake, and protein after social media-based nutrition education in rural and urban areas (Zaki *et al.* 2019). A study among young women in Pontianak showed that nutrition education on Facebook significantly increased knowledge of anemia, protein consumption, iron consumption, and vitamin C consumption among participants (Khotimah 2019).

A systematic review by Lroche *et al.* discovered that social media is possibly considered a possible means of communication to promote healthy lifestyle habits in organizations; however, several authors have recommended additional research into this technology to evaluate the incremental impacts of social media and promote healthy lifestyles (Laroche *et al.* 2020).

Some of the differences between this study and other studies are in the educational methods and strategies used. This study employed a hybrid learning approach, essentially a combination of constrained face-to-face education and education on social media. The nutrition pocketbook for pregnant women and the food monitoring card were used as self-assessment tools to assess daily nutrition practices. Meanwhile, online education was conducted by sharing nutritional information in the form of flyers on social media, such as WhatsApp, structurally and systematically.

The hybrid learning education method is most suitable applied during the pandemic, such as the current Covid-19 pandemic. It requires only limited direct contacts of individuals to prevent disease transmission. Therefore, the health services, particularly nutrition education can be accessed by pregnant women with less anxiety. Essentially, such strategy model can also be utilized in normal conditions in the future once the pandemic ends to optimize the restricted resources and costs. This can help increase the reach of educational materials through alternative communication means, such as social media. Despite its rigorous sampling and systematic approach in nutrition education, this research only involved limited populations, with a limited number of samples. The duration of the educational intervention was short for only three months, the number of direct sessions was only twice, and the remaining 12 sessions were conducted on the WhatsApp group. Consequently, the educational impacts on pregnancy outputs, such as Hb levels and MUAC might not be insufficient. Future research must improve the design of online education models, especially the frequency of message delivery, the length of intervention time, and a larger number of simples. Moreover, further research should improve the message content and delivery modes. It is suggested however that the District Health Office and related agencies necessarily change their policies of nutrition education strategies by combining face-to-face methods and using social media as a means of delivering nutrition education messages systematically and structurally to ensure sustainability.

CONCLUSION

Nutrition education did not significantly increase the mean hemoglobin and MUAC levels but reduced the prevalence of anemia in pregnant women in the intervention group. Thus, a structured education program for mothers during pregnancy can help improve the application of nutrition and health practices to prevent anemia and CED during pregnancy. The District Health Office and community health centers are expected to develop a structured educational model with a contextually appropriate and beneficial messages, strategies to harvest the benefit from the advent of information technology.

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DECLARATION OF INTERESTS

There was no conflict of interest among the authors.

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The Effect of Combined Extracts of Sappan Wood (*Caesalpinia sappan* L.) and Gotu Kola (*Centella asiatica* L.) in Improving Diabetic Condition in Rats

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ABSTRACT

This study aimed to determine the efficacy of combination of sappan (secang) wood and gotu kola extracts in reducing insulin resistance and Malondialdehyde (MDA) levels in diabetic rats induced by Streptozotocin (STZ) 65 mg/kg Body Weight (BW) and Nicotinamide (NA) 230 mg/kg BW. Forty-two male Sprague Dawley rats weighing ±200 g were divided into 7 groups: 1) control, 2) glibenclamide 0.45 mg/kg BW, 3) sappan wood extract (CS) 250 mg/kg BW, 4) gotu kola extract (CA) 500 mg/kg BW, 5) 1st combination of extracts of sappan wood and gotu kola (CSCA1) 125 mg/kg BW + 750 mg/kg BW, 6) 2nd combination (CSCA2) with 250 mg/kg BW + 500 mg/kg BW, and 7) 3rd combination (CSCA3) with 375 mg/kg BW + 250 mg/kg BW. The insulin resistance levels were measured using the HOMA-IR index based on fasting blood glucose and insulin. The Thiobarbituric Acid Reactive Substance (TBARs) method was used to measure MDA levels. All measurements were taken before treatment, 14 days after treatment, and 21 days after treatment. The group receiving CSCA3 showed significant reduction in insulin resistance (-3.32±0.05) and MDA levels (-2.04±0.37 nmol/ml) on Day 21 after treatment. The CSCA3 treatment did not show statistically different result compared to glibenclamide treatment ($p>0.05$). Hence, CSCA3 treatment was considered as the best proportion of sappan wood and gotu kola extracts mixture and the result is comparable to glibenclamide. This study shows that the combination of sappan wood and gotu kola extracts has the potential to be developed as a functional drink for people with diabetes.

Keywords: *centella asiatica*, combined extracts, diabetes mellitus, malondialdehyde, sappan wood

INTRODUCTION

An increase in blood glucose levels caused by impaired insulin production, insulin resistance, or both, as well as carbohydrate, lipid, and protein metabolism abnormalities, is the most prevalent symptom for Diabetes Mellitus (DM) (World Health Organization 2019). A survey by the International Diabetes Federation revealed that Type 2 Diabetes Mellitus (T2DM) accounts for 90% of all cases of diabetes. Diabetes causes approximately 4.2 million deaths among individuals aged 20 to 79 years old in 2019. The global prevalence of diabetes is expected to be around 463 million people (9.3%) in 2019 and it will rise to 700.2 million people (10.9%) in 2045, which is a 51% increase (International Diabetes Federation 2019).

Diabetes causes macrovascular problems such as cardiovascular disease, peripheral vascular disease, and cerebrovascular disease leading to morbidity and mortality. Diabetes also frequently causes microvascular problems such as retinopathy, nephropathy, neuropathy, chronic disease, and diabetic ulcers (Silva *et al.* 2017). All of these health issues lower the quality of life and productivity of the human resources, therefore causing increasing burden of health care costs and lowering workforce productivity both for the patient and their caretaker in a country.

Insulin Resistance (IR) is one of the main pathogenesis of T2DM and it is frequently undiagnosed due to the lack of physical symptoms when it arises. A cohort study by Wang *et al.* (2020) found a stronger association of diabetes incidence among adults in China with insulin resistance than with pancreatic beta cell dysfunction. The body is unable to utilize the insulin in hyperglycemia condition caused by disruption of insulin receptors. The

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pancreas then compensates for the loss of insulin synthesis, resulting in hyperinsulinemia. T2DM development later results in pancreatic beta-cell dysfunction and decreased insulin production (Saisho 2015). Thus, IR is an important marker for T2DM diagnosis and treatment.

The development of T2DM is also affected by oxidative stress. Hyperglycemia causes an increase in free radicals, particularly the type of Reactive Oxygen Species (ROS) in all body tissues. The presence of high free radicals in the body can cause lipid peroxidation in cell membranes, resulting in the formation of Malondialdehyde (MDA). MDA is a carcinogenic secondary product that is more persistent than other aldehydes, so it is the ideal indicator for oxidative stress on lipids (Ayala *et al.* 2014).

Metformin and sulfonylureas (glibenclamide, glycuidone, glicazide, and glimepiride) are two oral anti-diabetic medications that T2DM patients in Indonesia regularly take. However, they often suffer from side effects of these medications such as nausea and hypoglycemia (Putra *et al.* 2017). So, there is a need to develop innovative treatments for T2DM that have fewer side effects, are less toxic, and inexpensive.

High level antioxidant activity has been found to have a therapeutic impact for T2DM. Antioxidants serve in the body's defense system, trapping oxidants, producing inflammatory mediators, repairing damaged molecules, and beginning and enhancing endogenous antioxidant production (Adwas *et al.* 2019). Antioxidants are required to improve insulin sensitivity and oxidative stress conditions.

Sappan wood (*Caesalpinia sappan* L.) and gotu kola (*Centella asiatica* L.) are known as natural ingredients proven to be effective in treating T2DM due to their strong antioxidant capacity. Both of the plants have been used as functional drinks for people with T2DM (Badan Pengawasan Obat dan Makanan 2016; Fitriyanti *et al.* 2020). Many people combine plants or other ingredients that have a synergistic effect or are more potent than a single ingredient in traditional drink recipes to reduce side effects. However, there has been no research reported on the effect of combination of sappan wood and gotu kola as two natural components on DM. Therefore, to fill the gap of knowledge the research team analyzed the effect of combined extracts of sappan wood and gotu kola on fasting blood glucose, insulin levels, insulin resistance, and MDA levels in diabetic rats induced with STZ and NA. The researchers expected that this study could show the potentials of combination of sappan wood and gotu kola as a functional drink in improving T2DM conditions as compared to functional drink without the combination of both extracts.

METHODS

Design, location, and time

The study design was a randomized control group pretest-posttest design. The material origin and extraction process located in Materia Medika Batu, Malang City, Indonesia. The research on experimental animal was carried out at The House of Experimental Rats, Center for Food and Nutrition Studies, UGM, DIY Yogyakarta. The research was carried out from June to July 2021 with ethical clearance by The Research Ethics Committee of Faculty Medicine, Universitas Sebelas Maret No 36/ UN27.06.6.1/ KEP/EC/2021 and ID: 01/02/04/38.

Materials and tools

The materials for the treatment were sappan wood and gotu kola extracted with 96% ethanol as solvent. Modeling of hyperglycemia was done by inducing Streptozotocin (STZ) dissolved in citrate buffer and Nicotinamide (NA) dissolved in saline. Fasting Blood Glucose (FBG) was measured by the Glucose GOD FS Kit (DiaSys, Germany). Insulin levels was measured by mouse insulin ELISA Kit (FineTest, China). H_3PO_4, TBA, methanol, distilled water, and 1,1,3,3-Tetraethoxypropane (TEP) were used for measurement of MDA levels.

The tools used in this study were rotary evaporator, reciprocating shaker, syringe, oral gavage, microhematocrit, waterbath, cooling bath, centrifuge, microplate reader, cuvette and Uv-Vis spectrophotometer.

Procedures

Extraction of sappan wood and gotu kola. Sappan wood and gotu kola were washed and dried before being mashed and processed into simplicia. The extraction method used was maceration. Sappan wood was diluted with 96% ethanol as solvent in a ratio of 1:9 for 120 hours, while gotu kola was diluted with a ratio of 1:5

for 96 hours and stirred at room temperature. The amount of solvent was adjusted to the simplicia and must be completely submerged. Then, each was filtered with filter paper. The filtrate was evaporated at speed of 100 rpm and temperature of 60°C. The evaporation of sappan wood and gotu kola took 4 hours and 2 hours, respectively. The evaporation was stopped when the solvent was no longer dripping and the extract has thickened. The result of extracts was thick liquid with a yield of 10.71% from 700 g of sappan wood simplicia, while the extract of gotu kola leaves obtained a yield of 15.71% from 700 g of gotu kola simplicia.

Animal study. Total number of samples in this study was 42 male white rats (*Rattus novergicus*), Sprague Dawley strain. The rats were 8−10 weeks old and weighed ±200 g. The rats were acclimatized for 7 days under standard animal housing conditions (with the temperature was controlled at 25±2°C and maintained with 12 light-dark cycle) and were given standard AD II feed of 10−20 g and *ad libitum* water. T2DM modeling for all rats was done by intraperitoneal injection of 230 mg/kg BW of NA dissolved in 2 mg/200 g BW of saline. After 15 minutes, 65 mg/kg BW of STZ dissolved in 2 mg/200 g BW of citrate buffer was also injected (Muhlishoh *et al.* 2019). Hyperglycemic conditions (>150 mg/dl) were obtained within 72 hours (Ghasemi *et al.* 2014). Then, randomization was done where the rats were divided into 7 groups; each group was given different treatments: distilled water (Control), Glibenclamide 0.45 mg/kg BW (Glibenclamide), sappan wood extract (CS) 250 mg/kg BW, gotu kola extract (CA) 500 mg/kg BW, combination of sappan wood and gotu kola extracts 1 (CSCA1) 125 mg/kg BW + 750 mg/kg BW, combination 2 (CSCA2) 250 mg/kg BW + 500 mg/kg BW, and combination 3 (CSCA3) 375 mg/kg BW + 250 mg/kg BW. The doses of CA (500 mg/kg BW) and CS (250 mg/kg BW) in this study were based on previous studies by Fitrianda *et al.* (2017) and Sakir and Kim (2019) that showed a significant and same effect. The combination doses based on trial. The treatment was administered by using oral gavage for rats for 21 days. The Animal handling during treatment was the same as during the acclimatization phase.

Blood was drawn through the eye vein (orbital sinus) using the retro-orbital plexus method 4 times: before injecting STZ and NA, before treatment (0 day), 14 days and 21 days after the treatment. Microhaematocrit was scrapped on the medial canthus (under the eyeball towards the foramen poticus) and rotated 4 times to injure the plexus. Then, the blood was centrifuged at 1,000 rpm ±10 minutes at 40°C to obtain the supernatant/serum.

Fasting blood glucose (FBG) levels measurement. FBG levels were measured by the GOD-PAP (Enzymatic Calorimetric Test of Glucose Oxidase Phenol 4-Aminophenazone). The method applied was according to Subiyono *et al.* (2016).

Insulin levels measurement. Insulin levels were checked by reacting serum with monoclonal anti-mouse insulin (antibodies) that had been coated in microplate wells and the reagents provided in the mouse insulin ELISA kit. The procedure for analysis was performed following the protocol specified for the kit (FineTest, China).

Insulin resistance measurement. The assessment of insulin resistance was based on the FBG levels and insulin levels. Assessment of insulin resistance used a simple method using the HOMA-IR calculation formula (Fitriyanto *et al.* 2020).

$$\text{HOMA-IR} = \frac{\text{fasting blood sugar level (mg/dl) x fasting insulin level } (\mu g/l)}{405}$$

Malondialdehyde (MDA) levels measurement. MDA levels were measured with serum as a sample, standard, and blank using the Thiobarbituric Acid Reactive Substance (TBARs) method according to the method used by (Zainuddin *et al.* 2019).

Data analysis

All data obtained were presented as mean and standard deviation. Analysis was done by using SPSS (IBM, version 23) with one-way ANOVA statistical test, followed by post-hoc Tukey HSD test and Games-Howell test with a significant value of $p<0.05$.

RESULTS AND DISCUSSION

The results of this study indicate that there is a significant effect of the combination treatment of sappan wood extract and gotu kola in decreasing fasting blood glucose levels, and insulin resistance based on HOMA-IR index, and MDA levels as well as an increase in insulin levels in T2DM rats.

Fasting blood glucose levels. Table 1 shows that fasting blood glucose levels in all groups before STZ NA induction were normal. Fasting Blood Glucose (FBG) levels experienced a significant increase in all groups after STZ-NA induction (before each group was treated). This proves the success after 72 hours of STZ (65 mg/kg bw) and NA (230 mg/kg bw) induction. STZ and NA cause delayed onset of diabetes through β cell damage and immunologic reactions. STZ is a diabetagonic agent that causes damage to pancreatic β cells while NA aims to protect the cytotoxic effects of STZ (Szkudelski *et al.* 2013). NA is a derivative of vitamin B3 (niacin) which functions to increase the concentration of NAD+ or partially inhibit PARP-1 (Kishore *et al.* 2017). The data between groups before or and on day 0 after STZ-NA induction did not differ significantly, indicating the success of randomization and the results were considered homogeneous.

The results showed that all treatments groups experienced a significant decrease in FBG levels after 14 and 21 days of treatment, except for the control group. After 21 days of treatment, there was a much greater decrease in FBG levels closely to normal FBG levels (before STZ-NA induction) than the decrease at 14 days after treatment. Blood glucose levels did not decrease in the control group because STZ inhibited the Krebs cycle so that ATP production in the mitochondria was limited and continuously reduced pancreatic β cell nucleotides as reported by (Szkudelski *et al.* 2013).

The combination treatment showed that the CSCA1 treatment was not significantly different from the CA treatment. CSCA2 treatment was also not significantly different from CS in reducing FBG levels after 14 days of treatment. However, after 21 days of CSCA2 treatment the FBG levels was significantly reduced compared to CS, CA, or CSCA1. While CSCA1 treatment still showed no difference with CA, even though it was given for 21 days. This shows that the proportion of CSCA1 is not better in reducing FBG levels than the treatment without the combination (CA or CS). CSCA3 treatment showed the most reduction in FBG levels among other treatments. CSCA3 treatment for 21 days decreased the FBG levels (69.73%) more than 14 days of treatment (61.69%) in the control group. CSCA3 treatment was not significantly different from Glibenclamide after 14 and 21 days of treatment. This shows that CSCA3 treatment

Table 1. The effect of combined extracts of sappan wood and gotu kola on fasting blood glucose levels

Group	Mean±SD (mg/dl)			
	Before STZ-NA	Day 0	Day 14	Day 21
Control	70.81±1.04	267.92±3.33	271.01±3.91[d]	273.39±3.51[e]
Glibenclamide	72.46±2.24	267.26±5.01	103.89±3.73[a]	84.04±4.29[a]
CS	70.22±2.62	265.22±4.36	137.03±3.44[b]	114.76±3.79[c]
CA	70.22±2.88	267.86±5.30	152.39±4.09[c]	127.50±3.63[d]
CSCA1	69.23±2.84	264.98±4.17	146.82±3.69[c]	122.44±2.74[d]
CSCA2	70.42±3.24	264.80±3.88	135.60±3.76[b]	101.36±2.63[b]
CSCA3	70.81±2.54	264.98±4.78	103.82±3.10[a]	82.73±2.06[a]
p	0.529	0.698	0.000	0.000

CS: Sappan wood extract 250 mg/kg BW; CA: Gotu kola extract 500 mg/kg BW; CSCA1: Combination of sappan wood extract 125 mg/kg BW and gotu kola extract 750 mg/kg BW; CSCA2: Combination of sappan wood extract 250 mg/kg BW and gotu kola extract 500 mg/kg BW; CSCA3: Combination of sappan wood extract 375 mg/kg BW and gotu kola extract 250 mg/kg BW. Mean values with different superscript letters (a, b, c, d, e) within a column are significantly different ($p<0.05$) based on ANOVA and Tukey's post-hoc test is the best combination proportion with the a comparable ability as Glibenclamide in reducing FBG levels in T2DM rats.

This decrease in FBG levels occurs because the bioactive compound of the two ingredients that are thought to be effective as antidiabetic substances. It is caused by the positive effect of antioxidants such as flavonoids in both ingredients. Flavonoids are the most popular compounds for antioxidants because of their ability to breakdown free radicals and modulate signals to several cells. Flavonoids work by activating the Phosphoinositide 3-kinase (PI3K/AKT) pathway, inhibiting gluconeogenesis, and stimulating glycogen synthesis. Flavonoids work by activating the synthesis and translocation of Glucose Transporter Type 4 (GLUT4), increasing hexokinase activity in the liver, reducing the occurrence of pancreatic β cell apoptosis, activating PPARϒ expression to improve glucose uptake, activating the AMPK pathway, inhibiting tyrosine kinase activity, and activating NF-kB (Al-Ishaq *et al.* 2019).

Gotu kola also has main compounds that have potential as antioxidants, especially its asiaticoside and asiatic acid. Thipkaew *et al.* (2012) reported that asiaticoside has an antioxidant effect on neuropathy in diabetic rats. Another study reported that asiatic acid also works as an antidiabetic agent through increased glycolysis by restoring the activity of enzymes such as hexokinase, Glucose-6-Phosphate Dehydrogenase (G6PDH), and pyruvate kinase and found a decrease in glycogen in the liver in STZ-induced diabetic rats (Ramachandran & Saravanan 2013).

Insulin levels. All treatment groups experienced a significant increase in insulin secretion both at 14 and 21 days after treatment, except in the control group (Table 2). Without any treatment, the function of pancreatic β-cells in the control group is disrupted so that they are unable to produce enough insulin to compensate for insulin resistance (Saisho 2015).

The combination treatment showed that CSCA1 was not significantly different from CA after 14 and 21 days of treatment. CSCA2 treatment was also not significantly different from CS in terms of increasing insulin level. This showed that CSCA1 treatment was not better than CA and CSCA2 treatment was not better than CS treatment. On the other hand, CSCA3 treatment was able to increase insulin levels the most after 14 days (22.34%) and 21 days of treatment (31.09%) compared to the control group. The result form CSCA3 treatment was

Table 2. The effect of combined extracts of sappan wood and gotu kola on insulin levels

Group	Mean±SD (pg/dl)		
	Day 0	Day 14	Day 21
Control	421.74±6.89	$417.38±6.09^{a}$	413.92±5.66a
Glibenclamide	426.47±5.50	$515.38±5.91^{e}$	$547.01±5.74^{d}$
CS	425.56±8.21	$484.65±8.12^{cd}$	$514.65±8.40^{c}$
CA	428.47±7.75	$476.29±7.68^{bc}$	$499.01±8.92^{b}$
CSCA1	421.20±4.91	$469.01±5.84^{b}$	$488.83±7.12^{b}$
CSCA2	421.20±4.40	$492.11±6.38^{d}$	$524.65±4.29^{c}$
CSCA3	424.65±6.04	$510.65±6.42^{e}$	$542.65±5.70^{d}$
p	0.323	0.000	0.000

CS: Sappan wood extract 250 mg/kg BW; CA: Gotu kola extract 500 mg/kg BW; CSCA1: Combination of sappan wood extract 125 mg/kg BW and gotu kola extract 750 mg/kg BW; CSCA2: Combination of sappan wood extract 250 mg/kg BW and gotu kola extract 500 mg/kg BW; CSCA3: Combination of sappan wood extract 375 mg/kg BW and gotu kola extract 250 mg/kg BW. Mean values with different superscript letters (a, b, c, d, e) within a column are significantly different ($p<0.05$) based on ANOVA and Tukey's post-hoc test not significantly different from Glibenclamide treatment. This means CSCA3 treatment is the best proportion of combination from both extracts and it has comparable result to Glibenclamide in increasing insulin levels.

Impaired Insulin Receptor Substrate (IRS) production, decreased the GLUT-4 translocation, and glucose oxidation cause a decrease in insulin levels, thus glucose unable to enter cells and remains in circulation (hyperglycemia) (Lee 2006 in Nurhidajah & Nurrahman 2017). Increased insulin levels are thought to be because of a positive effect on insulin sensitivity, thereby the increasing secretion of insulin after treatment. Compounds that play a role in insulin secretion, especially through the Calcium (Ca) pathway, are flavonoids (Al-Ishaq *et al.* 2019). The increase in Ca ions in the cytoplasm of pancreatic β-cells will cause insulin secretion.

Brazilin is the main ingredient in sappan wood, member of flavonoid group and works specifically in inhibiting protein kinase C and insulin receptor serine kinase which plays a role in the regulation of insulin signaling. Brazilin induces GLUT4 from intracellular storage areas to plasma membrane via PI3K activation without affecting protein and GLUT4 synthesis (Nirmal *et al.* 2015). Asiatic acid has also been shown to increase insulin secretion by enhancing the PI3KT/Akt signaling pathway. Moreover, these compounds also improve glucose response by increasing muscle protein GLUT-4, IRS, IRS-1, and IRS-2 as reported by Ramachandran and Saravanan (2013 & 2015).

Insulin resistance. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) is an insulin resistance biomarker that uses fasting plasma glucose and insulin concentration to determine insulin sensitivity. The HOMA-IR is simple, efficient, and validated for evaluating insulin resistance against DM. The study results showed that all groups, except the control group, experienced a significant decrease in HOMA-IR after 14 and 21 days of treatment (Table 3). The results in the control group are not different from the result in a study conducted by Wang *et al.* (2020) which reported that there was no significant difference in the HOMA-IR index in patients with duration of T2DM of less than 1 year and patients with duration of T2DM of 30 years.

Table 3. The effect of combined extracts of sappan wood and gotu kola on HOMA-IR index

Group	Mean±SD (nmol/ml)		
	Day 0	Day 14	Day 21
Control	8.37±0.23	8.38±0.24^{d}	8.38 ± 0.21d
Glibenclamide	8.44±0.24	3.96±0.14^{a}	3.40±0.16^{a}
CS	8.36±0.24	4.92±0.19^{b}	4.37±0.20^{bc}
CA	8.50±0.30	5.37±0.21^{c}	4.71±0.18^{c}
CSCA1	8.26±0.17	5.10±0.12^{bc}	4.43±0.08^{c}
CSCA2	8.26±0.11	4.94±0.08^{b}	3.94±0.12^{b}
CSCA3	8.33± 0.10	3.92±0.08^{a}	3.32±0.05^{a}
p	0.423	0.000	0.000

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; CS: Sappan wood extract 250 mg/kg BW; CA: Gotu kola extract 500 mg/kg BW; CSCA1: Combination of sappan wood extract 125 mg/kg BW and gotu kola extract 750 mg/kg BW; CSCA2: Combination of sappan wood extract 250 mg/kg BW and gotu kola extract 500 mg/kg BW; CSCA3: Combination of sappan wood extract 375 mg/kg BW and gotu kola extract 250 mg/kg BW. Mean values with different superscript letters (a, b, c, d, e) within a column are significantly different ($p<0.05$) based on ANOVA and Tukey's (day 14) and Games Howell (day 21) post-hoc test

The combination treatment showed that the CSCA1 and CSCA2 treatments were not significantly different from the CA and CS treatments after 14 days and 21 days of treatment. This shows that the proportion of the combination of CSCA1 and CSCA2 is not better in reducing the HOMA-IR index than the treatment without the combination (CA and CS treatment). The treatment that showed the ability to reduce the HOMA-IR index the most was CSCA3. This treatment was not significantly different from Glibenclamide treatment after 14 and 21 days. CSCA3 treatment, compared to the control group, was able to reduce HOMA-IR as much as 61.69% after 14 days of treatment and 69.73% after 21 days of treatment.

High HOMA-IR index indicates disruption of the uptake and use of glucose by the body cells, resulting in an increase in blood glucose levels. In this study, the increase in insulin ability was thought to be caused by the repair response of target cells (muscle, adipose, and liver) to activate the use of glucose in cells as indicated by resistance values based on the HOMA-IR index which decreased after treatment. CSCA3 treatment showed the most optimal decrease in insulin resistance values and was not significantly different from Glibenclamide.

Malondialdehyde levels. The MDA levels of all groups showed a significant decrease after 14 days and 21 days of treatment, except the control group (Table 4). The control group after 14 days of treatment did not show a significant difference, in fact, there was a significant increase after 21 days of control treatment. This is thought to be due to the occurrence of excessive metabolic stress as a result of the development of T2DM conditions through several metabolic pathways; polyols, hexosamines, Advanced Glycation End Products (AGEs), and Protein Kinase Activation (PKC). Moreover, an increase in MDA levels proves that STZ-NA induction increases the ROS levels. This happens through a shift in the balance of redox reactions due to changes in carbohydrate and lipid metabolism which in turn will increase the formation of ROS from glycation reactions and lipid oxidation. Thereby reducing the antioxidant defense system (Halliwell & Gutteridge 2015). STZ is a source of free radicals, damaging DNA and causing cell death. A study reported a

Table 4. The effect of combined extracts of sappan wood and gotu kola on MDA levels

Group	Mean±SD (nmol/ml)		
	Day 0	Day 14	Day 21
Control	9.61±0.30	9.84±0.28[e]	10.06±0.27[e]
Glibenclamide	9.28±0.34	3.63±0.44[a]	2.32±0.24[a]
CS	9.04±0.43	5.48±0.53[bc]	3.91±0.16[c]
CA	8.51±0.38	6.84±0.23[d]	4.70±0.20[d]
CSCA1	8.88±0.25	6.36 ±0.13[c]	3.90±0.18[c]
CSCA2	9.26±0.53	5.18± 0.15[b]	3.25±0.46[b]
CSCA3	9.49±0.30	4.45±0.22[a]	2.04±0.37[a]
p	0.082	0.003	0.000

MDA: Malondialdehyde; CS: Sappan wood extract 250 mg/kg BW; CA: Gotu kola extract 500 mg/kg BW; CSCA1: Combination of sappan wood extract 125 mg/kg BW and gotu kola extract 750 mg/kg BW; CSCA2: Combination of sappan wood extract 250 mg/kg BW and gotu kola extract 500 mg/kg BW; CSCA3: Combination of sappan wood extract 375 mg/kg BW and gotu kola extract 250 mg/kg BW. Mean values with different superscript letters (a, b, c, d, e) within a column are significantly different ($p<0.05$) based on ANOVA and Games Howell (day 14) and Tukey's (day 21) post-hoc test significant increase in MDA levels and decreased endogenous antioxidant enzyme activity after STZ induction (Husna *et al.* 2019).

The results showed that the CSCA1 and CSCA2 treatments were not significantly different from the treatment without the combination, namely, CS treatment after 14 days of treatment. However after 21 days of treatment, CSCA2 significantly reduced MDA levels compared to CS and CSCA1. These results indicate that the proportion of CSCA1 combination is not better at reducing MDA levels than the treatment without the combination, namely, CS. The CSCA3 treatment showed the most reduction in MDA levels among the other treatments and showed comparable results to Glibenclamide treatment at both 14 and 21 days after treatment. This indicates that the proportion of CSCA3 treatment is the best combination of sappan wood extract and gotu kola for lowering MDA levels, and is similar to Glibenclamide. CSCA3 treatment for 21 days suppressed MDA levels (60.38 %) more than the control group's and (53.22 %) more at 14 days treatment.

Bioactive components of CSCA3 treatment are thought to be more effective in lowering the production of free radicals like ROS and other oxidants, inhibiting lipid peroxidation, and improving pancreatic cell injury in diabetic rats than other treatments. Brazilin has anti-inflammatory properties that prevent the formation of NO and iNOS (Nirmal *et al.* 2015). According to the study in metabolic syndrome rats by Pakdeechote *et al.* (2014), increased bioavailability of asiatic acid helped reduce ROS and other proinflammatory cytokines. Furthermore, it is claimed to be able to inhibit lipid peroxidation and a number of proinflammatory cytokines due to an increase in FFA in T2DM patients. Lower MDA levels indicate inhibition of lipid peroxidation. Muchtaromah *et al.* (2016) found that antioxidant overload increased MDA levels in diabetic rats, suggesting that an effective dose and duration of administration is required.

CONCLUSION

The combination of sappan "secang" wood extract and gotu kola had a significant effect in reducing insulin resistance and MDA levels in the rat models. The CSCA3 treatment, which was a combination of sappan wood extract 375 mg/kg BW, and gotu kola extract 250 mg/kg BW, was selected as the optimal dose to improve the condition of diabetic rats. Administration of CSCA3 for 14 days resulted in significant decrease in insulin resistance with decrease of HOMA-IR index by 61.69% and MDA levels by 53.22% as compared to the control group. More prominent decrease occurred on Day 21 with a decline of 69.73% for HOMA-IR and 60.38% for MDA level as compared to the control group. This study shows that combination of sappan wood and gotu kola has a potential to decrease oxidative stress and increase insulin secretion as well as insulin sensitivity in T2DM better than the administration of each extract alone without combination.

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DECLARATION OF INTERESTS

The authors declare that there is no conflict of interest with the parties involved in this research.

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An Additional Adequate Water Intake Increases the Amniotic Fluid Index in Pregnant Women with Oligohydramnios: A Systematic Review

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ABSTRACT

This systematic review aimed to answer whether an additional amount of water intake can increase the Amniotic Fluid Index (AFI) in pregnant women with oligohydramnios. Article searches were conducted and data was obtained from “SCOPUS”, “EBSCO”, “PUBMED”, “COCHRANE” and “Google Search” databases using the following keywords: “hypovolemic”, “dehydration” “pregnancy” “outcome”, “hydration”. “water intake”, “oligohydramnios”, and “amniotic fluid index”. We used MeSH headings (hydration pregnancy) for search keyword, Inclusion criteria were subjects who were pregnant women with oligohydramnios (without any pathological disorder in the mother and fetus), the outcomes include AFI of <5 cm, study design was prospective cohorts and clinical trials, consumption of plain water (non-calorie beverages) and language restriction applied for articles published in English. Out of 391 articles, eight articles that met these criteria for anaylsis. Result showed that additional amount of water intake for pregnant women with oligohydramnios without maternal/fetal abnormalities in the third trimester (28−37 weeks) can increase AFI. Oral maternal hydration gave a better effect than intravenous maternal hydration on AFI. The additional amount of water intake per day required by pregnant women with oligohydramnios to increase AFI to normal ranges from 1,500 to 2,500 ml depending on the condition of each pregnant woman. Additional water intake via oral can be a strategy for oligohydramnios therapy in pregnant women.

Keywords: amniotic fluid index, oligohydramnios, pregnant women, water

INTRODUCTION

Water intake is extremely essential for maintaining body homeostasis. Inadequate water intake may cause dehydration, which can bring unpleasant impacts (Subudhi *et al.* 2012). The European Food Safety Authority has suggested that each person should consume adequate water intake and has recommended daily water intake of 2,500 ml and 2,000 ml for men and women, respectively, in order to maintain urine osmolality at 500 mOsmol/kgH^2O (EFSA 2010; El-Sharkawy *et al.* 2015).

Studies have shown that hydration improvement through oral and/or intravenous routes can improve Amniotic Fluid Index (AFI) in pregnancy (Borges 2011). However, the minimum amount of additional daily water intake for the prevention of oligohydramnios for pregnant women is still unclear.

A study on urban women residing in the Indonesian capital, Jakarta, showed that 52.6% experienced dehydration and the lack of water intake can cause oligohydramnios (Mulyani *et al.* 2021). Oligohydramnios is a clinical condition when the amniotic fluid volume is <500 ml or AFI of <5 cm or <5 percentile of gestational age or a single deepest pocket of <2 cm (Chauhan *et al.* 2018). In general, the prevalence of oligohydroamnios in pregnant women is 3−5%, and it commonly occurs in the third trimester (Aggarwal & Patra 2018). A study conducted in

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the low-middle income countries suggested that oligohydroamnios occurs at the incidence of 1 out of 150 pregnancies, and it is strongly associated with dehydration during pregnancy (Figueroa *et al.* 2020).

Pregnant women requires extra fluid as there are physiological changes and fetal growth to maintain. Physiologically, there is intra- and extracellular fluid retention in pregnant women to ensure good metabolism during pregnancy (Ekpenyong *et al.* 2020). Studies have demonstrated adequate hydration intervention in pregnant women may improve oligohydroamnios, either by oral or intravenous hydration (Rawat *et al.* 2015; Lanni *et al.* 2007).

Evidences have shown that adequate hydration therapy in pregnant women could improve conditions in oligohydramnios. A review based on four studies in 2009, regarding the correlation between water intake and oligohydramnios have supported this hypothesis. (Hofmeyr *et al.* 2010). However, it has not revealed which method or route to insert water (oral or intravenous) has more impact on AFI? and how much additional drinking water is sufficient to correct oligohydramnios? Based on the description above, this review aimed to answer the question: can additional water intake improve oligohydramnios (AFI)?

METHODS

This review is based on the following hypotheses and conceptual framework: Does additional water intake could improve oligohydramnios; if benefits are obtained, what is the recommended additional amount of water intake and how is it administered to pregnant women with oligohydramnios? (Figure 1). This review method was a quantitative systematic review which include studies that have numerical data.

Design, location, and time

The design of this study was a systematic review based on published articles during the last 15 years (2006−2021), using PRISMA reporting guidelines. This study will follow the following guidelines: 1) establishing eligibility criteria; 2) establishing information sources; 3) selecting the study; 4) establishing the data collection procedure; and 5) establishing the data item selection

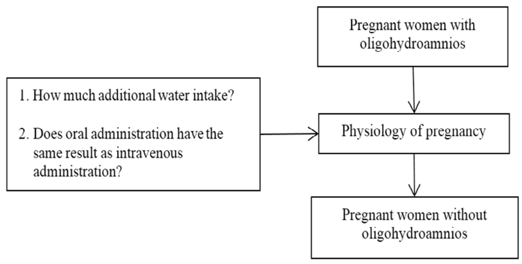


Figure 1. Conceptual framework

(Rethlefsen *et al.* 2021). Figure 2 illustrates the steps involved in performing a systematic review. The data source was secondary data, in which the data were obtained from results of previous studies. The sources of the data fulfilled inclusion criteria that had been determined earlier by the investigators. The Inclusion Criteria (IC) for this study included: 1) original and peer-reviewed research written in English, 2) pregnant women with oligohydroamnios (AFI of <5 cm) without any pathological disorder in the mother and fetus, 3) the design of the studies were clinical trials or prospective cohorts, and 4) consumption of plain water (non-calorie beverages). Only articles written in English were selected.

Study selection

The following four phases comprised the study selection process, as suggested by Rethlefsen *et al.* 2021: 1) Data were extracted

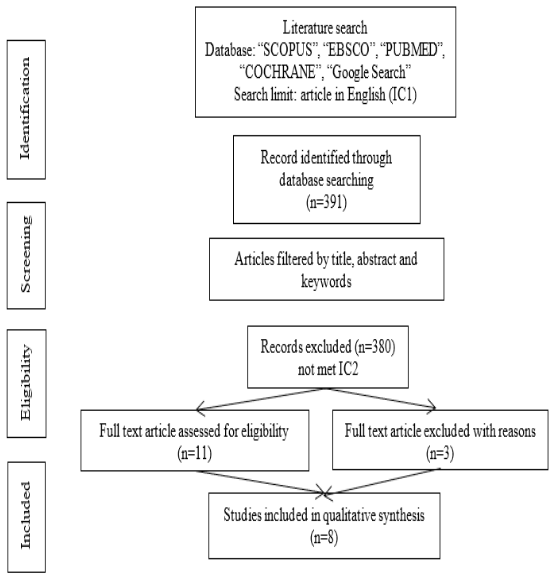


Figure 2. PRISMA flow diagram

by searching manuscripts from "SCOPUS", "EBSCO", "PUBMED", "COCHRANE", "Google Search" databases using the following keywords "hypovolemic", "dehydration" "pregnancy" "outcome", "hydration". "water intake", "oligohydramnion","amniotic fluid index"; 2) The title, abstract, and keywords of detected publications were analyzed and selected based on their eligibility requirements; 3) Complete or partial readings of the articles that were not removed in the previous phases were done to determine their suitability for inclusion in the review based on the eligibility criteria; 4) The reference lists of the publications were scanned to identify pertinent studies, and this phase was initiated with Phase 2.

The seven authors collaborated on these phases through an iterative process of author evaluations. Thus, any disagreements among the seven authors were discussed until a unanimous agreement was obtained. The summary table for this review was created manually from each selected study, using a data extraction form that included the following information: journal author, year, study setting, participant, research methods, study result, and conclusion. Each author evaluated publications that might be of relevance. The assessment procedure included reviewing both the complete text and the extracted data. These keywords resulted in the acquisition of 391 articles. However, only eight articles met the criterion for inclusion (Table 1).

RESULTS AND DISCUSSION

Results

Eight manuscripts met the criteria, which were included in the journal analysis. Table 2 presents a summary of eight selected studies including study design, sample size, intervention, results, strengths and weaknesses of each study. The obtained results of the studies discussed about oligohydroamnios in the third trimester (gestational age ranging from 28 to 37 weeks) of pregnancies.

The results showed that there were six articles discussed and compared oral and intravenous administration of water; and two articles discussed only oral administration in pregnancy. Regardless, the routes and the types of water administration, the result of each of eight studies show that water administration had an effect on improving oligohydramnios to be normo-hydramnios, which is measured by AFI.

All studies measured the quantity of additional water intake given to oligohydramnios pregnant woman with oligohydramnios whose gestational age ranging from 28−37 weeks. The result show that the quantity of additional water intake given to pregnant woman was between 1,500 ml to 2,500 ml per day. The duration of the water administration was from two hours until seven days, with the routes of administration through oral or intravenous.

In eight studies the water given were plain water and isotonic solution in four studies. The number of water administrated through oral ranging from 1,500 to 2,500 ml, and through intravenous ranging from 1,500 to 2,000 ml. All subjects in all studies with no pathological condition except oligohydramnios.

All of these studies have consistently provide evidences that oral hydration therapy for pregnant women with oligohydroamnios during the third trimester can increase AFI. Moreover, an oral hydration has better effect than intravenous

Table 1. Search strategies

Databases	Search strategies	Found	Used
SCOPUS	"hydration pregnancy" [MeSH Terms] OR ("preg-nant"[All Fields] AND "oligohydram-nios" [All Fields]) OR " amniotic fluid index" [all fields]	124	4
EBSCO	"hydration pregnancy" [MeSH Terms] OR ("preg-nant"[All Fields] AND "oligohydram-nios" [All Fields]) OR " amniotic fluid index" [all fields]	120	3
PUBMED	"hydration pregnancy" [MeSH Terms] OR ("preg-nant"[All Fields] AND "oligohydram-nios" [All Fields]) OR " amniotic fluid index" [all fields]	45	1
COCHRANE	"hydration pregnancy" [MeSH Terms] OR ("preg-nant"[All Fields] AND "oligohydram-nios" [All Fields]) OR " amniotic fluid index" [all fields]	7	1
Google search	Pregnancy and oligohydramnios, pregnancy and amniotic fluid index, hydration in preg-nancy, water intake in pregnancy	95	2

Tabel 2. Descriptive analysis of trials included in the systematic review

Authors & year	Study setting	Methods	Results	Conclusions
Lorzadeh *et al.* 2008	Design: Clinical trial. Subjects: 80 patients with low AFI and gestational ages greater than 35 weeks were studied without any maternal complications. AFI 5 cm, full-term gestational age greater than 35 weeks with intact amnion, maternal age between 15 and 38 years, women with parity of one to four Criteria for exclusion: Complications in the mother, including as hypertension, diabetes mellitus, cardiovascular, and hyperthyroidism; proven pre-eclampsia, verified fetal anatomical abnormalities as determined by ultrasound, and membrane ruptures.	Patients were randomized into four groups, which were: 1) Oral hydration group of 2,000 ml/2 hours; 2) Isotonic solution group of 2,000 ml/2 hours given by intravenous route (IV); 3) Hypotonic solution group of 2,000 ml/2 hours given by IV route; 4) Control group. Maternal AFI is determined prior to and following hydration treatment.	The increase in mean AFI following hydration treatment was significantly greater in the oral hydration group ($p<0.0001$), but not in the IV isotonic or IV hyptotonic groups compared to the control group.	In comparison to other groups, maternal hydration via oral water consumption is more beneficial. Numerous prior research have been undertaken to determine the effect of hydration therapy on AFI in oligohydroamniotic women. However, the majority of studies had a small sample size and studied pregnant women for a short length of time (gestational age >35 weeks). Additional studies with a large sample size and a wide range of gestational ages are needed to confirm the validity of this method in pregnant women with oligohydroamnios who are 35 weeks pregnant and have oligohydroamnios in order to avoid premature labor induction and serious consequences for mothers and their neonates.
Umber 2010	Design: Quasi experimental study. Subjects: 50 pregnant women with oligohydroamnios in the third trimester (AFI<5cm) were recruited prospectively. Inclusion criteria: Singleton pregnancy, full-term gestational age, intact amnion, absence of complications (moderate to severe anemia, heart disease, renal disease, moderate to severe pre-eclampsia or hypertension, or diabetes), absence of fetal morphological abnormality, and absence of symptoms of fetal distress.	The 50 patients were randomly assigned to one of two groups using a probability sampling technique (the intravenous hydration group and oral hydration group). The specific gravity of maternal urine and AFI were evaluated prior to and following hydration therapy. Within two hours, the intervention group received intravenous hydration therapy in the form of 2 liters 5% D/W. The group receiving oral hydration therapy was directed to consume 2,000 ml of water within two hours.	Hydration therapy improves amniotic fluid volume in pregnant women in the intravenous hydration group (altered mean AFI from 4.5 cm±1.25, CI:4.00−5.00; $p<0.05$), but not in the oral hydration group (altered mean AFI from 4.3 cm±1.23, CI:3.80−4.79; $p<0.05$). However, the percentage of mean AFI in the intravenous hydration group was 58.6%, which was not significantly greater ($p>0.05$) than the percentage of mean AFI in the oral hydration group, which was 58.2% In both groups, maternal hydration is associated with a decrease in urine specific gravity.	Although both intravenous and oral hydration enhance AFI in pregnant women with oligohydramnios, neither appears to be more effective at increasing amniotic fluid volume, and both may be beneficial in the management of oligohydroamnios (equal effect was found for oral and IV routes). This study suggests that hydration therapy can enhance amniotic fluid index and perinatal outcomes in patients with oligohydroamnios. Indeed, the outcomes may have detrimental implications related with oligohydroamnios.
Akter *et al.* 2012	Design: Randomized controlled trial. Subjects: 64 pregnant women with gestational age of 32 to 35 weeks within a year period in order to identify maternal hydration using oral route water drinking in subjects with oligohydroamnios with AFI<5 cm.	The women in the study were randomly assigned to one of two groups. Group A (intervention group): Patients were told to consume 2,000 ml water within two hours, followed by an additional 2,000 ml water each day for seven days. Subjects in Group B (control group) were permitted to drink water as normal. AFI was determined in both groups after two hours, 24 hours, and seven days of oral hydration treatment.	Mean AFI before therapy was 4.77 cm±0.42 (mean±SD) vs. 4.80 cm±0.43 (mean±SD), and post-therapy AFI was 6.35 cm±0.65 vs. 4.81 cm±0.42 after two hours, and 7.08 cm±0.21 vs. 5.0 cm±0.20 after 7 days, respectively, in the oral hydration and control groups. Labor occurs in 53.1% vs. 12.4% of women between 37 and 40 weeks, normal labor with vaginal birth occurs in 71% vs. 21.8%, caesarian section occurs in 29% vs. 78. 2%, and low birth weight infants occur in 12.5% vs. 81.25% in the intervention and control groups. Between intervention and control, the healthy fetus outcome was 87.1% vs. 59.4%, dyspnea was 12.9% vs. 50%, and perinatal death was 3.22% vs. 21.8%.	Maternal oral hydration therapy has significantly increased AFI, reduced caesarean section rate and increased fetal outcomes.

Continue from Table 2

Authors & year	Study setting	Methods	Results	Conclusions
Patrelli *et al.* 2012	Randomized controlled prospective study comparing pregnant women with idiopathic oligohydroamnios (group A, 66 women) against pregnant women without oligohydroamnios (control group, 66 women - group B, 71 women)	Oligohydramnios was identified using a 5 cm AFI. A 3.5-MHz convex probe was used for sonography. Daily intravenous infusions of 1,500 ml isotonic solution were administered to Group A for six days. AFI, non-stress test, and fetal bio-physical profile measurements were made on day 0 and day 7. Group A was subdivided into A1 and A2 subgroups. The subgroup A1 received 1,500 ml of oral hydra-tion per day, while the grouping A2 received 2,500 ml per day.	In general, no significant difference existed between the two groups. During recruitment, the mean AFI SD was 39.68±11.111 mm in group A, but 126.92±10.59 mm in group B (p<0.001). On day 7, the mean AFI in group A was 77.70±15.03 mm, whereas there was no change in group B. The mean AFI at birth was 86.21±16.89 mm in subgroup A1, but 112.45±14.92 mm in subgroup A2 (p<0.001). There was no statistically significant difference in fetal and neonatal outcomes between the control and study groups, as measured by the APGAR score, the pH of the umbilical artery at birth, and the necessity for transfer to a neonatal intensive care unit.	The study clearly demonstrated that pregnancies with oligohydroamnios who received acute phase intravenous hydration therapy for six days had significantly increased amniotic fluid volume compared to pregnancies without complication at the same gestational age who did not receive hydration therapy during the same time period. The study's data indicate that intermittent oral hydration therapy of 2,500 ml/day considerably raised amniotic fluid volume, resulting in normal AFI at birth. Only by randomly assigning group A to two subgroups with varying daily oral hydration protocols (i.e. 1,500 ml in subgroup A1 and 2,500 ml in subgroup A2) were the investigators able to determine critical and novel elements, namely the volume of daily drinking water intake required to achieve optimal performance. Indeed, even when both groupings reported an increase in AFI following medication, 2,500 ml daily oral intake may result in a higher rise in AFI than 1,500 ml daily oral intake. The study's primary limitation may include its small sample size.
Zafar *et al.* 2017	Design: Randomized controlled trial. Subjects: 80 pregnant women with gestational age of >28 weeks, AFI of <5cm, singleton pregnancy and intact membrane	Subjects were randomized and categorized into two groups of 40 people in each group. Group A received extra water intake of 2,000 ml in addition to their daily intake for seven days and group B was the group with daily usual hydration and their water intake was determined based on their sensation of thirst. AFI was measured before and after hydration therapy in 48 hours and one week following the therapy.	The pre-treatment mean AFI was 3.45 cm±0.50 in group A and 3.40 cm±0.49 in group B (p=0.656). The post-treatment mean AFI in group A was 6.83 cm±0.81 and it was 5.05 cm±0.75 in group B (p=0.001).	There was a significant increase of AFI after additional oral hydration therapy compared to daily usual water intake in subjects with oligohydroamnios during their third trimester and the therapy can be used compared to the invasive technique.
Ali & Ahmad 2018	Design: A quasi-experimental study model Subjects: 45 pregnant women with oligohydroamnios and the women were categorized into three groups of 15 women in each group: the first group received Intravenous (IV) isotonic solution,	The form of therapies in the three group were: 1) IV isotonic solution (normal saline) of 2,000 ml/2 hours; 2) IV hypotonic solution (Ringer solution) of 2,000 ml/2 hours; 3) Oral water intake of 2,000 ml/2hours. The instruments used in the study were: maternal questionnaire,	AFI increased significantly from 0.35 cm±0.07 to 1.7 cm±0.5 in IV isotonic group; while AFI increased significantly from 0.37 cm±0.08 to 1.9 cm±0.9 in IV hypotonic group. Moreover, increased AFI was found more obviously from 0.37 cm±0.07 to 2.7 cm±0.8 in the oral hydration group. The maternal and fetal outcomes were not significantly different among the groups, but the increase was more obvious in the oral hydration group AFI	The study showed a good effect of oral hydration compared to intravenous route. Women with oligohydroamnios might have a better chance of cure using oral hydration therapy than intravenous fluid and might achieve better maternal and fetal outcome with oral hydration therapy compared with other methods in other groups.

Continue from Table 2

Authors & year	Study setting	Methods	Results	Conclusions
	the second group received hypotonic IV solution and the third group received drinking water.	sonography report to measure AFI in women with oligohydroamnios before and after hydration, fluid diagram and data form of mothers and their babies.	increased significantly in the IV isotonic group and IV hypotonic group. However, AFI increased more obviously in the oral hydration group. Outcomes: Mode of delivery and the incidence of PPH were not significantly different among the groups. Moreover, APGAR score of five and NICU care as neo-natal outcomes were not significantly different among the groups.	Oral hydration is recommended for pregnant women with oligohydroamnios until the labor. Limitation: It was an interventional study and therefore, it was difficult to perform randomization and there was no control group to compare the interventional effectiveness.
Zafar *et al.* 2020	Design: Randomized clinical trial. 38 pregnant women with gestational age of 28−36 weeks, AFI<5 m, singleton pregnancy and intact membrane.	Subjects were randomized and categorized into two groups and each group contained 19 subjects. Group A received water intake of 2,000 ml within two hours every day for one week and group B received 2,000 ml intravenous fluid within 24 hours for 1 week. AFI was measured before and after 48-hour hydration and one week later.	Baseline AFI before therapy was 3.316 cm±0.8368 in group A and it was 3.211 cm±0.8178 in group B (p=0.697). AFI after 48 hours for group A was 5.926 cm±0.4593 and it was 5.784 cm±0.4622 for group B (p=0.348). AFI after 7 days was 8.286 cm±0.6000 for group A and 7.868 cm±0.2810 for group B (p=0.014).	There was a significant increase of AFI after oral therapy compared to those receiving intravenous fluid. Oral hydration can be used instead of intravenous hydration because it is simple, safe and noninvasive method. It is also easily acceptable to the patient.
Malik *et al.* 2021	Comparative prospective survey design (trial pre-post two groups of oral and IV). There were 100 patients with singleton pregnancy (50 in each group) with an AFI of <5cm gestational age of 28−37 weeks.	Patients were randomly assigned to two groups (each with 50 patients) using a lottery system. Patients in group A were told to drink 2,000 ml water everyday for seven days, whereas those in group B were given 2,000 ml 5% D/W in addition to their normal fluid intake. In both groups, the amniotic fluid index was determined before and after hydration.	Improvement was reported in 39 (78%) of group A patients and 22 (44%) of group B patients following the experiment (p<0.001). The pre-hydration mean AFI was 4.79 cm±0.53 in group A and 4.87 cm±0.36 in group B (p=0.383). The post-hydration mean AFI in group A was 6.79 cm 1.22 and in group B was 5.97 cm 1.37 (p=0.002).	In women with oligohydroamnios, oral hydration therapy is more successful than intravenous hydration therapy in terms of the frequency of increased amniotic fluid volume during the third trimester.

AFI: Amniotic Fluid Index; PPH: Post-Partum Hemorrhage; APGAR: Appearance, Pulse, Grimace, Activity, Respiration; NICU: Neonatal Intensive Care Unit

fluid therapy on AFI. This study has demonstrated that non-calorie water intake of 1,500−2,500 ml/day through oral route within several hours for seven days during the third trimester of pregnancy could increase and achieve normal AFI.

Discussion

The present review supports the previous review by Hofmeyr *et al.* (2002) that both oral hydration administration and intravenous solution administration can increase AFI in pregnant women with oligohydramnios. Furthermore, the majority of eligible studies (five of eight study) reported information regarding the oral administration gave a better effect than intravenous maternal hydration on AFI in pregnant women with oligohyramnios. The amount of additional water intake of 1,500 to 2,500 ml/day during the third trimester of pregnancy with oligohydramnios is suggested in order to increase and achieve a normal AFI.

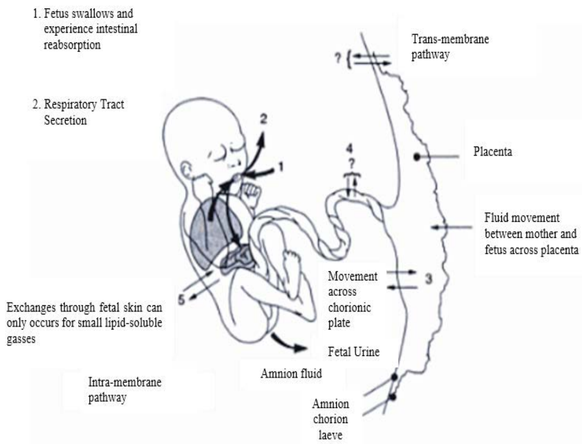
The body weight of women at full-term pregnancy increases approximately 12.5 kg, which consists of six to eight liters of extracellular fluid (intravascular and interstitial fluid) and the remaining is intracellular fluid. The interstitial fluid includes amniotic and placental fluid (Cunningham *et al.* 2014; IOM 2009). Water accounts for around 62% of total gain at term, fat accounts for 30%, and protein accounts for 8%; however, these figures vary considerably. The component of weight increase attributable to bodily water is the most variable. A positive correlation between increased total body water and infant birth weight has been documented (Kominiarek *et al.* 2017; Mulyani *et al.* 2021). At the early stage of pregnancy, there is reduced plasma osmolality, which causes reduced sense of thirst and secretion of antidiuretic hormones. (Carmody *et al.* 2009). Fluid requirements depend on energy intake, which is 1 to 1.5 ml fluid for each kilocalorie of energy intake. During pregnancy, there is increased mean energy expenditure of 300 kcal/day; therefore, a pregnant woman needs at least 300 ml additional water intake (one glass to two glasses). Furthermore, additional water intake is required for amniotic fluid, 500−1,200 ml (Indonesian Society of Obstetrics and Gynecology 2013).

Prerequisite for well intrauterine development of fetus and good neonatal outcome is adequate amniotic fluid. Available data suggests that hydration has advantages in increasing amniotic fluid volume in subjects with oligohydroamnios and normo-hydramnios. However, the mechanism of how maternal hydration could increase amniotic fluid volume still not yet understood. Five studies have been conducted to compare the effectiveness between oral and intravenous hydration and all of those studies reveal that oral route is more effective than the intravenous. The advantages of oral hydration are that it is easy, low budget, noninvasive and it does not need hospitalization or strict monitoring (Zafar *et al.* 2020). Kilpatrick and Safford reported that maternal hydration increases AFI, both in subjects with oligohydroamnios and normo-hydramnios (Kilpatrick *et al.* 1991; Kilpatrick *et al.* 1993). Fait *et al.* (2003) demonstrated that 75% of mothers with oligohydroamnios who consumed water of 2,000 ml/day have increased AFI as much as 50%. Increased hydration in pregnant women could speed up the mean uterine artery rate, which will result in increasing AFI; however, the mechanism of this increase has not been clearly identified. The increase may be caused by increased perfusion of uterine placenta.

Amniotic fluid is mostly created by fetal urine during the second trimester of pregnancy and is eliminated via fetal swallowing. Additionally, amniotic fluid is absorbed through the fetal lungs and placenta. When nearing full-term pregnancy, maternal hydration and osmolarity have an effect on the volume of amniotic fluid, which has an effect on the production and reabsorption of fetal urine (Figure 3 and Figure 4). Shahnazi *et al.* (2012) demonstrate that increased water intake in pregnant women with oligohydroamnios will increase utero-placental blood flow and increase the production of fetal urine, hence increasing the amniotic fluid volume.

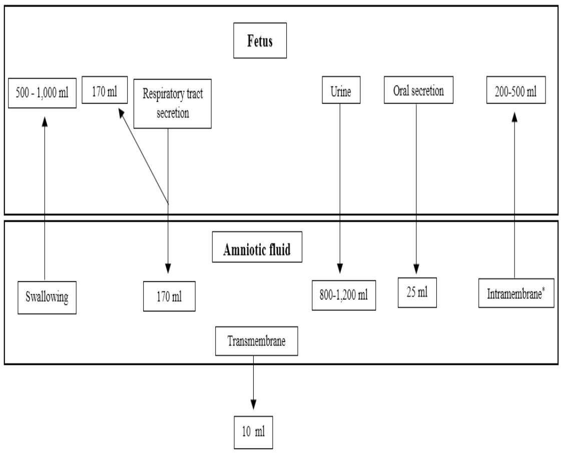
In adults, diuresis has direct correlation with osmolality and intravascular fluid volume. Based on clinical data, fetal could have a response to osmolality changes and maternal intravascular fluid volume. The production of fetal urine may alter with maternal osmolality changes. A previous study showed that improvement of utero-placental perfusion as a result of increased maternal plasma volume would increase fetal oxygenation (Shahnazi *et al.* 2012). Magann *et al.* (2011) demonstrated that amniotic fluid volume and AFI increase significantly after pregnant women experienced oral hydration.

Oligohydroamnios could cause complications in pregnancy and affects mother and fetus. Based on study by Wright *et al.* (2010), it is found that normal maternal hydration status could reduce prematurity rate, abortion rate and other complications, both for mother and fetus. A cohort prospective study by Mulyani *et al.* (2018) in Jakarta revealed that, there are differences in water intake levels between dehydrated and hydrated pregnant women. Mean body weight and length, head circumference, and chest circumference of the newborns from dehydrated pregnant women were lower than those from hydrated pregnant women (Mulyani *et al.* 2021).



Source: Brace (2004)

Figure 3. Movement of fluid flow into and out of the amniotic cavity



Source: Brace (2004)

Figure 4. Movement pathway of fluid flow into and out of the amniotic cavity

The results of the study have implications on the urgency of healthy hydration education such as amount of water intake, quality and safety of drinking water for productive age women, expectant mothers as well as pregnant women. The IOM determined the dietary reference value for total water consumption based on the median total water intake observed in NHANES III, which was 3,000 ml/day for pregnant women (IOM 2004). In terms of public health, according to the Ministry of Health of the Republic of Indonesia's decree number 28 of 2019, it is advised that pregnant women in Indonesia take between 2,450 and 2,650 ml of water per day, or the equivalent of approximately 10−11 glasses (MoH RI 2019). As with EFSA, the Indonesian Ministry of Health established dietary reference values based on a theoretical relationship between water and energy intake, recommending that between 1−1.5 ml of water should be consumed for each kcal of energy intake (Bardosono *et al.* 2017). The other reason is mean body weight and height of healthy adult female in calculating the DRI of water by IOM (2004) was 61 kg and 163 cm respectively; while for calculating DRI of water for Indonesian women by MoH RI was 55 kg and 159 cm. Assuming similar level of physical activity, at normal condition the lower the body weight the lower the requirement for energy and water, as such also applies to pregnant women.

Implementation of healthy hydration education recommendation at individual and clinical settings for pregnant women depends on various things such as body size, physical activity, gestational age, environmental temperature and health condition. For this purpose, healthy hydration education and training are important for healthcare professionals, productive age women, expectant mothers as well as pregnant women. Pregnant women with specific health problem should consult to healthcare professionals.

In addition, since the mechanisms of water intake for overcome oligohydramnios are not fully understood, further studies are required to identify the mechanism of hydration to increase AFI in pregnant women with various kind of pathological conditions as well as the minimum water requirement of each pathological condition.

CONCLUSIONS

Pregnant women with oligohydramnios without other maternal/fetal abnormalities in the third trimester (28−37 weeks) could increase AFI through drinking water. Oral hydration provides a better effect than intravenous hydration on AFI in pregnant women with oligohydroamnios. The additional adequate water intake for pregnant women with oligohydramnios in order to have the effect of increasing and achieving a normal level of AFI, ranges from 1,500 to 2,500 ml or the equivalent of about six to ten glasses per day depends on the clinical condition of the pregnant woman. Healthy hydration educational sessions are necessary for healthcare professionals, productive age women, expectant mothers as well as pregnant women. Especially, for pregnant women this education should stress on the amount of water intake required for pregnant women to prevent the development of oligohydroamnios.

Further studies are required to identify the mechanism of hydration to increase AFI in pregnant women with various pathological conditions. It is crucial to conduct a review about the effects of the quality of drinking water of pregnant women on fetal and neonatal growth and development, with emphasize on linear growth and cognitive function.

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DECLARATION OF INTERESTS

There is no conflict of interest in this present study.

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Economic and Consumption Variables and Their Associations with Stunting Prevalence: A Provincial Analysis of the Indonesian Child Nutritional Status Survey 2019

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ABSTRACT

The study aims to analyze the relationship between economic and food consumption variables with stunting prevalence among Indonesian children. The unit of analysis for this cross-sectional study was secondary data set from 2019 for 34 provinces obtained from Statistics Indonesia, the Food Security Agency, and the Ministry of Health of the Republic of Indonesia. In the majority of provinces (88.24%) the stunting prevalence was still categorized as serious public health problem with the prevalence of 30% or higher. The economic outlook in 2019 showed an economic growth, decrease in Gini ratio and the unemployment rate in 34 provinces. However, the poverty rate was very diverse between provinces and concentrated in eastern Indonesia. There were 17 provinces that had higher food than non-food expenditures. There were 31 provinces with normal energy adequacy level and 28 with normal protein adequacy level. However, there was no provinces reached maximum score in Desirable Dietary Pattern (DDP) as the indicator for food diversity, signaling that none of the provinces achieved adequate diversity in food consumption in 2019. The multivariate linear regression with backward elimination technique showed that seven of independent variables were qualified for the final model with R2 of 0.7406. The three variables significantly correlated with stunting prevalence ($p<0.05$) were food expenditure, protein adequacy level, and DDP score. Hence, these variables can be categorized as causal factors for stunting at provincial level analysis which can feed the food and nutrition policy and its monitoring and evaluation strategy. However, further analysis is needed to determine the direct and indirect relationship between economic factors, food expenditure, and food consumption with the prevalence of stunting among children in Indonesia so that stunting prevention and alleviation programs can be more precise and optimal.

Keywords: economic variables, food consumption, stunting

INTRODUCTION

Indonesia is still struggling with stunting as public health problem. In 2018, the stunting prevalence in Indonesia reached 30.8% in 2018 (MoH RI 2018) where a prevalence of above 30% is considered as serious public health problem (De Onis *et al.* 2019). This high prevalence is far from achieving the target from the Sustainable Development Goals (SDGs) of zero hunger in 2025.

WHO (2013) stated that childhood stunting causes short term and long term negative consequences. Globally, around 0.9 million from 1.4 million deaths in children under five years old are associated with stunting. The lack of nutrient lead to weaker immune system which causes the body to be more susceptible to disease, both infectious and non-communicable disease (Reinhardt & Fanzo 2014). The growth retardation is not only limited to physical aspects, stunting causes decrease in intellectual and cognitive functions which lead to lower academic achievement and productivity. In addition, childhood stunting is also associated with increase prevalence of obesity both in children or adults. The risk of obesity increases 2.4 times in children with stunting as they get older (WHO 2013; De Onis & Branca 2016). The decrease of productivity in Indonesia that was caused by stunting lead to 0.15−0.67% economic losses of Gross Regional Domestic Product (GRDP) in 2013 (Renyeot *et al.* 2016).

There is a strong association between stunting prevalence and income per capita in a country (Fenta *et al.* 2020). The Indonesian Economic situation has been marked by positive economic growth. However, this growth should

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also be assessed from other indicators such as the poverty rate, unemployment rate, and economic inequality to offer more comprehensive view.

Subramanyam *et al.* (2011) stated that good economic growth only benefits the high-income group and does not help people from the low-income quintile groups. This economic inequality is measured by Gini ratio. Gini ratio in Indonesia reached 0.38 in 2018 (BPS 2019) which shows an adequate equality. Black *et al.* (2013) stated that stunting prevalence in children under 5 years old in poor households is 2.47 times higher than stunting prevalence in rich households. In 2018, poverty rate in Indonesia, as one of developing countries, reached 9.66% (BPS 2019). Poor households in Indonesia are more vulnerable to stunting because of their limited access to food with adequate quantity and quality (Beal *et al.* 2018).

Increasing income without decreasing economic inequality and poverty rate is common in the developing countries and will influence the prevalence of stunting. (Rabbani *et al.* 2016). Low purchasing power will lead to consumption of cheap food with high energy density and lack of diversity for example, cereals and noodles. Long-term consumption of such food will cause micronutrient deficiency in children. While on the other hand, the proportion of nutrient rich food such as animal source foods, tend to decrease (DiSantis *et al.* 2013; Faradina *et al.* 2018).

Against these backgrounds, regarding the high stunting prevalence and its relationship to economic situation in various settings, it is important to fill the research gap on the assessment of economic situation and food consumption in Indonesia. This study also aims to analyze the economic variables associated with stunting based on the WHO stunting framework (2013).

METHODS

Design, location, and time

This study was a cross sectional analysis of secondary data set from 2019 regarding economic variables and food consumption at provincial level. Sources of data are Statistics Indonesia (BPS), Food Security Agency of the Ministry of Agriculture of the Republic of Indonesia (BKP), and Ministry of Health of the Republic of Indonesia. The data set used was from 2019 and provinces level (34) were chosen as the units of analysis, this decision was based on the completeness and availability of data that can be accessed through official online publication.

Data collection

Dependent variable in this study was stunting prevalence among children aged 0−59 months based on data from the 2019 Child Health Status Survey (SSGBI). Stunting prevalence in this publication is calculated based on the result of integration survey between National Socioeconomic Survey and Child Health Status Survey that were conducted at provincial and district levels in Indonesia. Meanwhile, independent variables that were used in this study were economic growth, economic inequality, unemployment rate, poverty rate, total of food expenditure, and food consumption. Sources of data are the 2019 data collections from 34 provinces in Indonesia. The selection of these data collections was based on the availability of data in national publication with data in provincial level as the main unit.

Economic growth was measured by the value of Gross Regional Domestic Product (GRDP). This GRDP data were collected from the publication of Statistics Indonesia, namely, “The Gross Regional Domestic Product of The Provinces in Indonesia by Business Field from 2016−2020”. The values of GRDP (IDR/cap/year) in that publication refer to the amount of added value produced by production units in part of a region in specified period of time (usually one year) and the production units are grouped into 17 categories of industry.

Economic inequality is measured by Gini ratio which collected from Statistics Indonesia publication, the Calculation and Analysis of Macro Poverty in Indonesia in 2019. Gini ratio’s formula is described as follows (BPS 2019).

$$G = 1 - \sum_{k=1}^{n}(X_k - X_{k-1})(Y_k + Y_{k-1})$$

where G is Gini ratio; X_k is cumulative proportion of population for k=0, 1, 2, … n with X0=0 and X1=1; Y_k is cumulative proportion of expenditure for k = 0, 1, 2 … n with Y0=0 and Y1=1.

Other economic variable in the analysis was poverty rate (%). The data for each province were collected from Statistics Indonesia publication, the Calculation and Analysis of Macro Poverty in Indonesia in 2019. The poverty rate was calculated based on this formula (BPS 2019).

$$P_\alpha = \frac{1}{n}\sum_{i=1}^{q}\left[\frac{z-y_i}{z}\right]^\alpha$$

where, α=0; z=poverty line; yi= the average of monthly expenditure/capita of population below the poverty line (i=1,2,3,…,q), yi<z ; q=sum of population that lives below poverty line; n=population.

Other independent variables were unemployment rate and food expenditure. Those data were collected from Statistics Indonesia publication, namely, Key Indicators of Indonesia Labor Market 2019 and Executive Summary of Consumption and Expenditure of Indonesia 2019. Meanwhile, food consumption variables consisted of energy adequacy level, protein adequacy level, and Desirable Dietary Pattern (DDP) score. All data on food consumption variables were collected from publication of the Food Security Agency (BKP), the Food Consumption Development Directory 2020.

Energy and protein adequacy levels are the adequacy rate for each energy and protein consumption compared to the Indonesian Recommended Dietary Allowance (RDA). RDA for energy is 2,100 kkal/cap/day while RDA for protein is 57 gram/cap/day. The RDA is based on the average needs of Indonesia's population regardless of gender and age (MoH RI 2019). Desirable Dietary Pattern score is an indicator for assessing food intake diversity based on the energy balance obtained from various food groups in the diet. The food groups are divided into nine groups based on proportion of their energy consumption: 1) Cereals and grains 50%; 2) Tubers 6%; 3) Animal-source foods 12%; 4) Oils and fats 10%; 5) Fruit/seed oil 3%; 6) Nuts 5%; 7) Sugar 5%; 8) Vegetables and fruits 6%; 9) Others (seasoning and herbs) 3%.

The DDP score calculation is based on energy consumption at the population level. Energy proportion in food groups is multiplied by each rating of each food group, which is based on the function of food. The rating in each group is as follows: 1) The rating for cereals and grains, tubers, oils and fats, fruit/seed oil, as well as sugars is 0.5; 2) The rating for animal-source foods and nuts is 2; and 3) For vegetables and fruits the rating is five. The ideal score for DDP in each food group was obtained from multiplying the rating by energy proportion of each food group. If those DDP scores are summed, it will produce the DDP score of the regions with maximum score of 100. This score means that the quality of population's food consumption reaches the ideal level. Higher DDP score indicates a more diverse and nutritionally balanced food consumption in the region. The details of ideal DDP score in each food groups are as follows: 1) Cereals and grains (25); 2) Tubers (2.5); 3) Animal source foods (24); 4) Oils and fats 5; 5) Fruit/seed oil (1); 6) Nuts (10); 7) Sugar (2.5); 8) Vegetables and fruits (30); 9) Others (0) (BKP 2015).

Data analysis

Data in this study were analyzed using multivariate linear regression with backward elimination technique. The selected independent variables which were included in the final model were based on p-value of each independent variable ($p \leq 0.05$). Below is the complete model for the multivariate linear regression.

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_8 X_8 + \varepsilon$$

where Yi: Stunting prevalence; β_0: Slope; β_1, β_2…, β8: Intercept/regression coefficient of stunting; X1: Gini ratio; X_2: GRDP of region (IDR/capita/year); X_3: Unemployment rate; X_4: Poverty rate; X_5: An average of food expenditure (IDR/capita/year); X_6: Energy consumption (energy adequacy level as a percentage); X_7: Protein consumption (protein adequacy level as a percentage); X_8: Desirable Dietary Pattern score; ε: Error.

RESULTS AND DISCUSSION

The stunting prevalence in each province in 2019 was varied. However, there were no provinces with low prevalence or very low prevalence (De Onis *et al.* 2019). Overall, the Indonesian economic growth was still in line with the plotted scenario with the GRDP in each province has been increasing through the years. Unemployment rate and Gini ratio also decrease in all provinces. On the other hand, similar to stunting poverty is varied in 34 provinces.

Referring to the WHO framework for child malnutrition, economic situation is one of the contextual causes of stunting, while household expenditure is one of the causal causes. Household expenditure in almost all provinces is dominated by expenditure on food. This is also followed by improvement in quantity of food consumed. The average of energy and protein adequacy levels in

34 provinces in 2019 was categorized normal. However, DDP score, which indicates diversity of food consumption was low none of the 34 provinces reached the ideal score of 100.

Multivariate linear regression showed that seven of independent variables are able to explain 74.06% of stunting prevalence in the model. Three of those variables, which are food expenditure, protein adequacy level, and DDP score, significantly associated with stunting prevalence in 34 provinces.

Stunting prevalence situation in Indonesia. The stunting prevalence in Indonesia in 2019 was varied (Figure 1). There were 14 provinces that stunting were categorized as serious public health problem. Those provinces were Aceh, West Nusa Tenggara, West Kalimantan, South Kalimantan, Central Sulawesi, South Sulawesi, Southeast Sulawesi, West Sulawesi, Gorontalo, Maluku, Papua, East Nusa Tenggara, Central Kalimantan, and North Sumatera.

Figure 1 showed that category of prevalence threshold for stunting distribution is divided into three categories, which are very high (≥30%), high (20−<30%), and medium (10−<20%) (De Onis *et al.* 2019). There were 16 provinces that were categorized as provinces with high prevalence, which were South Sumatera, North Maluku, East Kalimantan, North Kalimantan, Central Java, East Java, West Sumatera, Bengkulu, West Java, Lampung, West Papua, Riau, Banten, North Sulawesi, Special Region of Yogyakarta, and Jambi. Meanwhile, four provinces were categorized as provinces with medium prevalence. There were no provinces with low prevalence (2.5−<10%) or very low prevalence (<2.5%) according to prevalence threshold for stunting.

Economic situation in Indonesia. In Indonesia, 97.06% of provinces were having improvement of economic growth in 2019. Table 1 shows that the GRDP rate was increasing positively. Central Sulawesi was the province that had the highest GRDP rate in Indonesia (7.28%), while Papua Province experienced a slowdown of GRDP rate which was marked by a negative value. The province with the highest unemployment rate in Indonesia was Banten Province (8.11%) while the lowest was Bali Province (1.57%).

Papua had the highest poverty rate (26.55%), while Special Capital Region of Jakarta (3.42%) and Bali (3.61%) had the lowest poverty rate in 2019 (Table 1). Kementerian PPN/Bappenas (2018) stated that poverty rate in Indonesia is influenced by topographic condition. The provinces that are located in the eastern part of Indonesia are bordered and separated by mountains and valleys and scattered as small island. This condition of isolation can hold up mobility of population, distribution of goods and services, as well as delivery of basic services to the community. Poverty rate in those several provinces can cause unequal income distribution that can lead to economic inequality.

The inequality of income distribution is measured by Gini ratio that ranges from 0, which represents perfect equality, to 1, which represents perfect inequality. Table 1 shows that the highest Gini ratio recorded in DI Yogyakarta (0.43) meanwhile, the lowest in Bangka Belitung (0.26).

Situation of food expenditure and food consumption in Indonesia. The positive economic growth in majority of provinces in Indonesia can lead to an increase in food expenditure at household level (Sekhampu 2012). Indonesia monthly expenditure per capita on food was IDR 593,450/capita/month in 2019. If monthly food expenditure per capita in each province was compared to national expenditure, 19 provinces

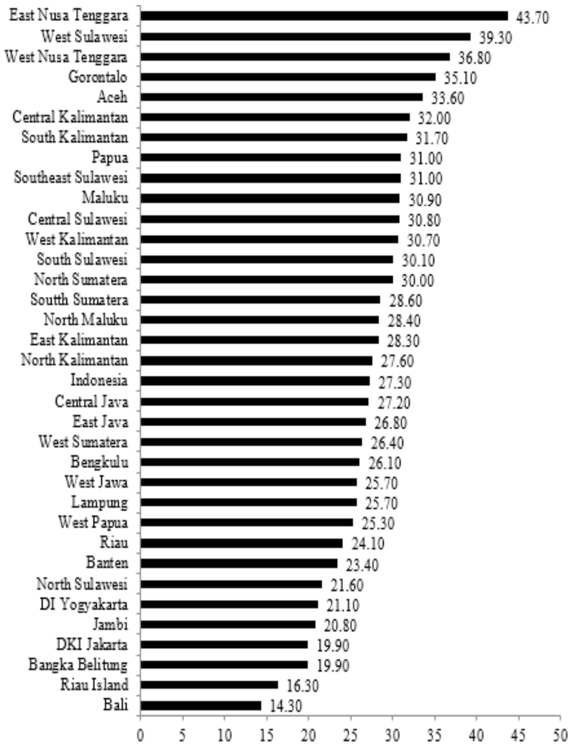

Figure 1. Stunting prevalence in 34 provinces in Indonesia in 2019

Table 1. Economic situation in 34 provinces in 2019

Name of province	Gini Ratio	GDRP Rate (%)	Poverty Rate (%)	Unemployment Rate (%)
Aceh	0.32	2.39	15.01	6.17
North Sumatera	0.32	4.15	8.63	5.39
West Sumatera	0.31	3.87	6.29	5.38
Riau	0.33	0.49	6.90	5.76
Jambi	0.32	2.80	7.51	4.06
South Sumatera	0.34	4.44	12.56	4.53
Bengkulu	0.33	3.44	14.91	3.26
Lampung	0.33	4.30	12.30	4.03
Bangka Belitung	0.26	1.31	4.50	3.58
Riau Islands	0.34	2.30	5.80	7.50
Special Capital Region of Jakarta	0.39	4.92	3.42	6.54
West Java	0.40	3.72	6.82	8.04
Central Java	0.36	4.71	10.58	4.44
Special Region of Yogyakarta	0.43	5.48	11.44	3.18
East Java	0.36	5.00	10.20	3.82
Banten	0.36	3.36	4.94	8.11
Bali	0.37	4.51	3.61	1.57
West Nusa Tenggara	0.37	2.73	13.88	3.28
East Nusa Tenggara	0.36	3.61	20.62	3.14
West Kalimantan	0.32	3.69	7.28	4.35
Central Kalimantan	0.34	3.99	4.81	4.04
South Kalimantan	0.33	2.57	4.47	4.18
East Kalimantan	0.34	2.70	5.91	5.94
North Kalimantan	0.29	3.18	6.49	4.49
North Sulawesi	0.38	4.70	7.51	6.01
Central Sulawesi	0.33	7.28	13.18	3.11
South Sulawesi	0.39	5.95	8.56	4.62
Southeast Sulawesi	0.39	4.49	11.04	3.52
Gorontalo	0.41	4.88	15.31	3.76
West Sulawesi	0.37	3.78	10.95	2.98
Maluku	0.32	3.70	17.65	6.69
North Maluku	0.31	4.15	6.91	4.81
West Papua	0.38	0.29	21.51	6.43
Papua	0.39	-17.16	26.55	3.51

Statistics Indonesia 2019; GDRP: Gross Regional Domestic Product had a monthly expenditure lower than the national average. The largest monthly food expenditure per capita was found in DKI Jakarta (IDR 979,228/capita/month), while the lowest was in West Sulawesi (IDR 435,527/capita/month) (Table 2).

Based on the proportion of expenditure, 22 provinces in Indonesia had a food expenditure proportion more than the national proportion which was 49.21% (Table 1). If it is viewed by food and nonfood expenditure category, there are 50% of provinces in Indonesia that had higher food expenditure than nonfood expenditure in their households. The highest proportion of food expenditure was East Nusa Tenggara (59.25%), while the lowest proportion of food expenditure was Special Region of Yogyakarta (38.62%).

Table 2 also presents the energy adequacy level, protein adequacy level, Desirable Dietary Pattern score (DDP) of each province. Province that had the highest energy adequacy level was South Kalimantan (113.50%) and the lowest was in North Maluku (84.00%). As for protein adequacy level, province that had the highest protein adequacy level was West Nusa Tenggara (133.30%) and the lowest protein adequacy level was Papua (79.10%). Based on the energy adequacy, 31 provinces had reached the normal category (90−120%). However, three provinces were still categorized as province with mild energy deficit (80−89%) which were Maluku, North Maluku, and Papua. As protein adequacy level, there were 28 provinces that were categorized as province with normal protein adequacy level. Meanwhile, one province experienced moderate protein deficit (Papua) and two provinces (Maluku and North Maluku) had mild protein deficit. Other three provinces were categorized as province with excess of protein intake with the percentages of more than 120%. Those provinces were Special Capital Region of Jakarta (123.90%), Special Region of Yogyakarta (124.00%), and West Nusa Tenggara (133.30%). Further, for DDP score. all provinces in Indonesia had not reached the ideal score, which is 100. The highest DDP score was found in Special Region of Yogyakarta, which was 94.40, and the lowest score, which was 65.90 in Papua.

Association of economic factors, food expenditure, and food consumption with stunting prevalence. There was one variable from the eight independent variables that was eliminated from the regression model, it was unemployment rate. The R2 value in the final regression model was 0.7406. This means that 74.06% of the variance of stunting prevalence was able to be explained by those independent variables that included in final model while 24.43% was presented by other independent variables outside this study. The final model of regression analysis is stated below.

$$Y=100.56859 - 8.16721X_1 + 0.00000219X_2 + 0.11342X_4 - 0.00003835X_5 - 0.16428X_6 + 0.50819X_7 - 1.05468X_8$$

According to the final model, there were seven independent variables that were included but only three of them were significantly associated with stunting prevalence ($p \leq 0.05$). They were food expenditure ($p<0.0001$), protein adequacy level ($p=0.0017$), and DDP score ($p<0.0001$) (Table 3). An increase of one Indonesian Rupiah (IDR) in food expenditure would have decreased a 0.000039% in stunting prevalence. Similarly, Breisinger and Ecker (2014) found that 0.9% improvement in food expenditure per capita can decrease 0.07% in child stunting.

Not only will food consumption be increasing in quantity because of the increasing food expenditure, it may also indicate better dietary diversity. Food diversity will fulfill nutrition needs optimally (Bloem *et al.* 2013). This is supported by the analysis where DDP score also had a significant association with stunting prevalence in 34 provinces in Indonesia in 2019. An increase of one point of DDP score would have decreased 1.06% prevalence of stunting. This is in line with Mahmudiono *et al.* (2017) study, which stated that diverse diet prevents stunting cases in East Java Province.

However, despite the positive association between DDP and decreasing stunting prevalence, Table 2 shows that DDP scores in 34 provinces had not reached the ideal score of consumption diversity. It indicates that the increase in income which increases the total food expenditure does not have the same impact on the diversity of food consumed. Improper allocation for food expenditure would decrease health and nutritional status, especially in children (Titaley *et al.* 2019). Such as shown a study in Egypt, where increase in food expenditure was spent more on consumption of junk food than nutritious food, especially for animal-source foods (Rashad & Sharaf 2018).

This study also found that an increase of 1% in protein adequacy level will increase

Table 2. Food expenditure and food cnsumption situation in 34 provinces in 2019

Name of province	Food expenditure*		Food consumption**		
	(IDR /cap/month)	%	Energy adequacy level (%)	Protein adequacy level (%)	DDP score
Aceh	576,093	55.35	98.10	103.40	72.40
North Sumatera	585,134	54.32	103.80	111.40	86.10
West Sumatera	635,738	51.77	101.50	102.80	81.40
Riau	618,112	48.76	98.60	102.40	84.00
Jambi	601,608	53.07	98.10	101.70	84.30
South Sumatera	517,928	52.05	101.10	105.30	84.40
Bengkulu	552,579	49.98	99.50	102.70	80.10
Lampung	498,535	51.81	97.80	100.90	86.90
Bangka Belitung	733,471	48.96	102.30	117.00	83.80
Riau Islands	805,732	46.58	101.50	114.90	84.60
Special Capital Region of Jakarta	979,228	42.17	104.40	123.90	88.20
West Java	635,623	49.13	104.70	113.80	86.10
Central Java	492,077	49.16	100.40	105.10	88.40
Special Region of Yogyakarta	557,239	38.62	104.90	124.00	94.40
East Java	523,677	49.44	101.60	106.80	88.40
Banten	735,398	50.42	107.10	117.60	86.20
Bali	650,604	43.14	107.60	115.80	89.10
West Nusa Tenggara	582,461	52.54	111.00	133.30	86.80
East Nusa Tenggara	474,377	59.25	93.70	96.40	69.20
West Kalimantan	587,654	52.78	91.10	98.20	76.30
Central Kalimantan	608,949	50.08	102.40	111.40	83.70
South Kalimantan	645,091	49.85	113.50	119.60	84.90
East Kalimantan	734,705	42.36	96.30	111.40	85.80
North Kalimantan	804,422	51.39	95.60	113.30	81.10
North Sulawesi	586,304	46.23	102.10	111.60	85.30
Central Sulawesi	490,256	49.57	96.10	99.90	82.20
South Sulawesi	520,889	49.82	102.10	111.70	84.80
Southeast Sulawesi	488,733	47.09	99.50	108.50	83.40
Gorontalo	485,745	46.77	100.30	109.40	80.50
West Sulawesi	435,527	51.4	101.20	104.00	78.80
Maluku	528,603	50.54	89.10	88.40	71.10
North Maluku	524,906	50.55	84.00	84.20	76.70
West Papua	695,602	51.57	95.90	98.00	80.80
Papua	686,431	54.14	88.80	79.10	65.90

*Statistics Indonesia 2019; IDR: Indonesian Rupiah; DDP: Desirable Dietary Pattern

Table 3. Results of multiple linear regression analysis

Variable	B	SE	p
Intercept	100.56859	17.30855	<.0001
Gini ratio	-8.16721	21.69622	0.7096
GRDP	0.00000219	0.00000112	0.0615
Poverty rate	0.11342	0.19309	0.5620
Food expenditure	-0.00003835	0.00000678	<.0001
Energy adequacy level	-0.16428	0.22007	0.4621
Protein adequacy level	0.50819	0.14528	0.0017
DDP score	-1.05468	0.19159	<.0001

B: Parameter Estimation; SE: Standard Error; $p \leq 0.05$; GRDP: Gross Regional Domestic Product ; DDP: Desirable Dietary Pattern

stunting prevalence by 0.51% in children aged 0−59 months in 34 provinces. This can be seen as a counter intuitive finding. However, it can be interpreted as an indicator of wealth inequality. The increase of animal-source food consumption as good quality protein only happened among upper middle and high-income groups. These groups can consume animal source food repeatedly with the maximum amount, which is 36.76% from total daily food consumption (Weatherspoon *et al.* 2017). In addition, data also shows that the distribution of protein consumption in Indonesia is dominated by grains (47.08%), the domination of grains to fulfill protein adequacy level with limited consumption of animal-source foods can cause the lack of micronutrient intake such as amino acid, iron, and other micronutrient which can lead to stunting (Ernawati *et al.* 2021). Similar paradox also shown in Mahmudiono *et al.* (2017) who stated that increased in fish consumption in East Java Province has 1.83 times risk of causing stunting in children. This is caused by the type of fish that is consumed is dried salted fish which is cheap but lack in nutrition. Headey *et al.* (2018) stated that consuming multiple animal-source foods is more advantageous than consuming any single animal-source food. Thus, food diversity and the quality of the food source of the nutrient is an important information to be gathered along with the quantitative calculation of nutrient adequacy alone, such as for energy or protein.

Vollmer *et al.* (2014) stated that stunting in children in developing country can be influenced by economic inequality which cause inequal food expenditure among the population. People living under the poverty line are unable to access quality food while on the other hand due to increasing income happening in the developing country, household expenditure is shifted toward non-food expenditure (Ghosh 2018). This phenomenon is described in Table 2 where 50% of provinces in Indonesia had greater proportion of non-food expenditure than food expenditure. These results supported the logic on the relationship between the causal factors such as food expenditure and food consumption with stunting (WHO 2013).

Rizal and van Doorslaer (2019) and Wicaksono and Harsanti (2020) stated that the economic growth and poverty reduction can reduce stunting prevalence among children in Indonesia. Economic equality will improve the access of poor mothers and children to effective health service and quality food consumption. In this study, the relationship directions between economic variables still cannot be identified. This becomes the limitation of this study where the path of direct or indirect relationship of economic and food consumption variables to stunting are unable to be evaluated further. The researchers suggest the use of path analysis to clarify the relation between economic variable and food consumption variable and stunting in Indonesia. Another limitation in this study is the use of provincial data as unit analysis which make generalization more difficult.

CONCLUSION

The 34 provinces had positive value of GRDP rate and decrease in unemployment rate in 2019. However, the poverty level varies with provinces in the eastern part of Indonesia had higher rate. This economic inequality was characterized by the Gini ratio ranged between 0.26−0.43.

Improvement in economic situation was followed by increasing household expenditures in 34 provinces. There were 50% provinces that displayed greater food than non-food expenditure in 2019. There were 91.2% provinces that were categorized as having normal energy adequacy level and 82.4% provinces that were categorized as having normal protein adequacy level. However, there were no provinces that had reached maximum score of DDP.

Multivariate regression analysis showed that total food expenditure, protein adequacy levels, and DDP score had a significant association with stunting prevalence. An increase of one rupiah in food expenditure will decrease stunting prevalence about 0.000039% and 1 point of DDP score will decrease stunting prevalence about 1.06%. However, an increase of 1% in protein adequacy level will increase the stunting prevalence about 0.51%.

Further analysis should be done to determine the path of relationship between economic variables, as contextual factor of stunting, and food expenditure and food consumption, as the causal factor of stunting prevalence in Indonesia. Path analysis will be able to categorize direct and indirect variables associated with stunting prevalence. The results of path analysis can feed into more precise and optimal stunting prevention and alleviation programs.

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DECLARATION OF INTERESTS

The authors have no conflict of interest to declare.

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