Jurnal Gizi Indonesia

(The Indonesian Journal of Nutrition)

VOLUME 11 NOMOR 2 JUNI 2023 ISSN 1858-4942

Artikel :

Diterbitkan oleh :

Departemen Ilmu Gizi Fakultas Kedokteran Universitas Diponegoro
Bekerjasama dengan
Persatuan Ahli Gizi Indonesia (PERSAGI) DPD Jawa Tengah

Jgizindo	Volume 11	Nomor 2	Halaman 77-139	Semarang Juni 2023	ISSN 1858-4942

P-ISSN: 1858-4942
E-ISSN: 2338-3119

Jurnal Gizi Indonesia

Indonesian Journal of Nutrition

Volume11 Nomor 2, Juni 2023

Susunan Tim Redaksi

Alamat Redaksi dan Tata Usaha : Departemen Ilmu Gizi, Fakultas Kedokteran, Universitas Diponegoro, Gedung Departemen Ilmu Gizi, Jln Prof. H. Soedarto, SH. Tembalang Semarang 50275, Telp/Fax. (024) 76402881. *Homepage* : http://ejournal.undip.ac.id/index.php/jgi/. Email jgi@fk.undip.ac.id

Jurnal Gizi Indonesia (*Indonesian Journal of Nutrition*) terbit dua kali setahun (Juni dan Desember) oleh Departemen Ilmu Gizi Fakultas Kedokteran Universitas Diponegoro bekerjasama dengan Persatuan Ahli Gizi Indonesia (PERSAGI) DPD Jawa Tengah.

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Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition)
Jurnal Gizi Indonesia
(The Indonesian Journal of Nutrition)
Vol. 11, No. 2, June 2023 (77-84)
Submitted: 11 April 2022, Accepted: 01 March 2022
Online https://ejournal.undip.ac.id/index.php/jgi

STUDY OF VITAMIN D3-FORTIFIED GOAT KEFIR ON PLASMA FIBRINOGEN LEVELS OF DIABETIC RATTUS NORVEGICUS RATS

Tania Masha[1], Astika Widy Utomo[3], Martha Ardiaria[1], Ahmad Syauqy[1,2], Ayu Rahadiyanti[1,2], Choirun Nissa[1,2], Mutiara Irma Maharani[1], Fairuz Zulfa[1], Binar Panunggal[1,2*]

ABSTRACT

Background*: Diabetes mellitus is often associated with the occurrence of complications. Haemostatic factors, especially hyperfibrinogenaemia, is a common cause of the complication. Goat kefir and vitamin D3 may act as an antioxidant and anti-inflammation agent which can repair pancreatic beta cells.*

Objectives*: This study aimed to analyse the effect of vitamin D3-fortified goat milk and plasma fibrinogen levels in diabetic rats.*

Materials and Methods*: This study was an experimental study with pre-post only group design. The samples were 21 male rats divided into four groups; negative control (K-), positive control (K +), treated with unfortified goat kefir (P1), and treated with vitamin D3-fortified goat kefir (P2). The 35-day intervention was conducted, the goat kefir dose was 2 ml/200 g BW/day and the vitamin D dose 600 IU. Fasting blood glucose and plasma fibrinogen were assessed pre- and post-intervention. Blood glucose level was evaluated by GOD-PAP method, while plasma fibrinogen was assessed by Enzyme-Linked Immunosorbent Assay (ELISA) method. The data were analysed with paired t-test and One-Way ANOVA.*

Results*: There were not significant difference levels of fibrinogen between groups. The intervention groups both showed an insignificant decrease of plasma fibrinogen. The plasma fibrinogen of group treated with vitamin D3-fortified goat kefir went down to 13.47 mg/dl from 16.49 mg/dl (p = 0.49). Meanwhile, the group treated with unfortified goat kefir showed a decrease from 26.81 mg/dl to 24.94 mg/dl (p=0.83). On the other hand, there was a significant decrease in fasting blood glucose in the group treated with vitamin D3-fortified goat kefir from 181.75 mg/dl to 116.25 mg/dl (p=0.03).*

Conclusion *Our results demonstrate that administration of vitamin D3-fortified goat kefir can decrease fasting blood glucose but not in plasma fibrinogen.*

Keywords *: Diabetes Mellitus; Fastin blood glucose; Fibrinogen; Goat kefir; Vitamin D3 Fortification*

BACKGROUND

Today, diabetes mellitus (DM) has become a global health issue. Type-2 diabetes reported higher prevalence compared to type-1 diabetes and other types of diabetes. According to the International Diabetes Federation (IDF) quoted by Basic Health Survey, the prevalence of diabetes in Indonesia will increase to 6.3% in 2030 from 5.1% in 2000 [1]. World Health Organization (WHO) reported that in 2000 there were 171 million diabetic people, and this will increase to 366 million in 2030 [2].

Diabetes mellitus (DM) is frequently undetected and only come discovered after complications occur [1]. The most common complications are cardiovascular complications. Cardiovascular complications contributed to 50% of death among people with type-2 diabetes [2]. Several studies have shown that haemostatic factors, especially hyperfibrinogenaemia, is associated with atherosclerosis and its complications [3]. Type-2 diabetes associated with peripheral arterial disease affects and increases the concentrations of fibrinogen. [4]

Fibrinogen act as an inflammatory marker that can develop due to the linkage between insulin resistance and vitamin D deficiency [5,6]. Vitamin D deficiency is correlated with metabolic syndrome, cardiovascular disorders, and type-2 diabetes mellitus. Vitamin D stimulates the expression of insulin receptors in peripheral tissues to increase glucose transport [8]. Also, increased insulin sensitivity in response to improved vitamin D status may be due to the suppression of chronic inflammation [9].

[1]Department of Nutrition, Faculty of Medicine, Universitas Diponegoro, Indonesia
[2]Center of Nutrition Research, Universitas Diponegoro, Indonesia
[3]Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Diponegoro, Indonesia
[*]**Correspondence** : panunggalbinar@live.undip.ac.id

e-ISSN : 2338-3119, p-ISSN: 1858-4942

Vitamin D3-fortification in food is needed to increase vitamin D intake among diabetic. Food fortification is an effort to add one or more nutrients that aims to prevent or correct nutritional deficiencies in the population [10]. Goat milk contains more protein, vitamin A, thiamine, riboflavin, niacin, pantothenate, calcium, phosphorus compared to cow's milk [7]. However, some vitamins in goat milk, such as vitamin B6, vitamin B12, vitamin C, vitamin E and folic acid is relatively low, but fermented goat milk had a value of vitamin D3 [7]. A study showed that the consumption of vitamin D3-fortified milk improves levels of 25(OH)D as an improvement in inflammation [11]. At the molecular level, vitamin D is needed to reduce oxidative stress and reduce insulin resistance [12]. Therefore, to increase the nutritional content of goat milk to provide health benefits and reduce inflammation, it must be fortified with vitamin D [7]. Vitamin D3 fortification in goat kefir is necessary due to its low content of vitamin D only at 0.08/100 g [13].

According to Indonesian Dietary Recommendation (AKG) 2019, the recommended dose of vitamin D for 1-64-year-old is 15 µg/day or 600 IU/day.15 Goat milk is an alternative choice for consumers who are allergic to cow's milk. However, the strong aroma and flavour of goat milk caused dome people to dislike it [15]. Besides, milk is easily damaged by microorganisms as it is an excellent growth medium for bacteria and potentially become a medium of spreading pathogenic bacteria. Therefore, to improve the quality and minimize that risk, it is necessary to carry out further processing by fermenting milk into kefir with the help of microbes [16].

Kefir is a product of fermented milk made by inoculating kefir seeds, which consist of bacteria and yeast. Kefir seeds contain natural probiotics, especially *Lactobacillus acidophilus, Bifidobacterium bifidum*, lactic acid bacteria, and yeast, which is an antibacterial, anti-inflammatory, antioxidant, antitumoral, antioxidant and probiotic [17]. In diabetic study showed goat kefir could improves blood glucose and some inflammatory biomarkers [18]. As a probiotic, kefir may weaken the acute phase inflammation characterised by fibrinogen level, which can improve risk factors of chronic diseases such as type-2 diabetes and coronary heart disease [19]. Also, kefir contains vitamin B, vitamin K, folic acid, minerals, amino acids, proteins that are easily absorbed [20.21]. Kefir, as a probiotic drink, may reduce fasting blood glucose and HbA1C in patients with type-2 diabetes mellitus [22]. The administration of 2 ml/day of kefir shows an increase in inflammatory mediators in diabetic rats [23].

Diabetes in rats was determined by measuring fasting blood glucose levels. Rats were declared diabetic if their glucose level exceeded 140 mg/dl [24]. Based on previous studies, daily administration of up to 2ml/200 g body weight of kefir significantly lowered plasma glucose and repair pancreatic β-cells among diabetic rat in the 35-day intervention [24]. As diabetes known as the risk factor for cardiovascular diseases characterised by an increase in plasma fibrinogen, people need to be cautious with this disease. The background of this study was the correlation between diabetes and the likeliness of vitamin D-fortified goat kefir to reduce inflammation. This study aims to determine the effect of administration kefir fortified goat milk with vitamin D3 on fibrinogen levels in diabetic *Rattus Norvegicus* rats.

MATERIALS AND METHODS

This study is an in-*vivo* experiment within clinical nutrition study with a true-experimental method. Pre-post only group design was used with Wistar rats as the sample and data were collected from December 2019 to January 2020. Vitamin D3-fortified goat kefir was made at Integrated Laboratory of Diponegoro University. The experiment was conducted at Animal Trial Laboratory, Faculty of Medicine, Diponegoro University. Fasting blood glucose was assessed at Regional Health Laboratory Hall, Semarang, while plasma fibrinogen was assessed at GAKI Laboratory, Diponegoro National Hospital. Male *Rattus norvegicus* (Wistar rats) were obtained from Farhan Mouse Farm, Semarang.

The sample size was determined using Federer formula, which resulted in seven rats in every group, a total sample of 28 rats. Wistar rats were chosen due to its physiological similarity to human, rapid metabolic rate, are not affected by oestrogen, high adaptability, can be easily cared for, tame, omnivorous, resistant to intervention and unable to vomit thus are easily controlled during the experiment. The dependent variable in this experiment is plasma fibrinogen, while the independent variable is the administration of vitamin D3-fortified goat kefir. The control variable is strain, age, body weight, diet, handling of rats, the environment in which the rats were placed, and also sanitation.

The experiment was started by preparing the rats diet, which was standard rats feed BR-2. The rats were *ad-lib* fed with the following calculation: 6% x body weight (g) = amount of feed (g)/day. They were also *ad-lib* watered. The rats were given seven days to adapt to their cage where temperature and humidity were kept around 25-27°C and 40-70% respectively. During this period, the 12:12 LD cycle was also kept to

e-ISSN : 2338-3119, p-ISSN: 1858-4942

help their adaptation process. The cages were cleaned, and the rats body weight was measured daily. After the adaptation period, rats were randomly grouped into four groups: negative control (K-), positive control (K+), given unfortified goat kefir (P1), and given with vitamin D3-fortified goat kefir (P2). The K+, P1 and P2 groups were injected with 230 mg/kg body weight of nicotinamide (NA), diluted in 0.9% NaCl via intraperitoneal. After 15 minutes, 65 mg/kg body weight streptozotocin (STZ) in citrate buffer (pH 4.5) was also injected. These doses are the maximum stable dose of diabetes to be observed in rats [24]. After 15 days of diabetes induction, sample rats were put in fasting for 12 hours. Blood was drawn from plexus retro orbitalis using haematocrit tube. Before and after 35 days of intervention, fasting blood glucose and plasma fibrinogen were assessed. Rats were declared diabetic if the blood glucose >140 mg/dl [24].

Few days after the intervention, a rat from the negative control group (K-) and three Wistar rats from intervention groups (P1 and P2) were found dead. Therefore, at the end of the experiment, there were six rats in the negative control group, seven rats in the positive control group, and four rats each in P1 and P2 groups.

Administration of goat kefir to rats was done based on the previous study that showed that administration of 2 mL/200 g body weight of goat kefir lowered plasma glucose and improved pancreatic β-cells in rats after 35 days. Intervention in this study was carried out for 35 days. The fortified and unfortified goat kefir was made every three days following instruction from the previous study [11].

The collected data contained rats body weight measured every week using a digital animal scale, blood glucose and plasma fibrinogen before intervention as pre-test data, and after intervention as post-test data. Fasting blood glucose was assessed using Glucose Oxidase-Peroxidase Aminoantipyrine (GOD-PAP). In contrast, plasma fibrinogen was determined using Rat Fibrinogen ELISA (Enzyme-Linked Immunosorbent Assay) kit read at 450 nm wavelength by Universal Microplate Reader ELX800.

The collected data was then tested for normality by the Shapiro-Wilk test. Differences on pre- and post- blood glucose and plasma fibrinogen were tested using paired t-test. The difference in all groups was tested by One-Way ANOVA or Kruskal-Wallis test depending on the normality of the distribution (One-Way ANOVA test normally distributed data). The confidence level of the analysis was 95%, or the significance level was 0.05 (p-value). This study has earned an ethical clearance (No. 04/EC/H/FK-UNDIP/I/2020) from the Health Research Ethics Commission (KEPK) Faculty of Medicine Diponegoro University/dr. Kariadi Regional Hospital, Semarang.

RESULTS

Table 1. Baseline Characteristics

Indicators	Groups				*p*
	K- (n = 6)	K+ (n = 7)	P1 (n = 4)	P2 (n = 4)	
Body weight (g)	276.8 ± 23.20	266.1 ± 29.00	255.8 ± 19.29	291.8 ± 18.84	0.202*
Fasting blood glucose (mg/dl)	96.98 ± 19.27[a]	155.18±16.33[b]	196.00±46.62[b]	181.75±40.31[b]	<0.001
Plasma fibrinogen (mg/dl)	17.58 ± 4.23	51.57 ± 57.62	26.81 ± 12.99	16.49 ± 6.29	0.089**

Value is shown as Mean ± SD
*One-Way ANOVA test **Kruskal-Wallis test
a, b) different notations in the same line showed significant differences in the post-hoc test

Body Weight Characteristics

The changes in body weight during a 35-day intervention is shown in Table 2. All groups showed comparable increase (p = 0.202). K- group showed an increase of 17.83 ± 26.32 g after the intervention. The increase in the control group indicates that glucose metabolism among that group was kept and the rats were healthy. The increase in other groups (K+, P1 and P2) showed diabetes with a sign of polyphagia, occurring because of the lack of glucose intake in cells stimulates the hypothalamus to increase appetite. The difference in body weight between groups after the intervention was significant, with a p-value of 0.034.

Characteristics of Fasting Blood Glucose

Table 2 shows the characteristics of fasting blood glucose levels before and after an intervention. The fasting blood glucose of control group K- and K+ before intervention (96.98 ± 19.27 mg/dl and 155.18 ± 16.33 mg/dl respectively) was increased after 35-day of intervention and there was no significant difference compared to blood glucose level after intervention (113.18 ± 8.59 mg/dl and 208.12 ± 103.24 mg/dl; p>0.05). Although the blood glucose level of the control group increased, the post-intervention mean value of the K-

group was not classified as diabetes mellitus. Meanwhile, P1 and P2 groups shows a significant decrease in blood glucose level pre- (196.00 ± 46.62 mg/dl and 181.75 ± 40.31 mg/dl) and post-intervention (114.35 ± 6.18 mg/dl and 116.25 ± 5.12 mg/dl; $p<0.05$). If we observed the four treatment groups, the highest difference in blood glucose level reduction (ΔGDP) was shown in the P2 group ($p=0.03$). This result indicates that the diabetic group treated with fortified goat kefir (P2) was able to reduce blood glucose level significantly compared to the diabetic group treated with unfortified goat kefir (P1).

Table 2. Characteristics of Body Weight, Blood Glucose, and Plasma Fibrinogen Pre- and Post-Intervention

Indicators	Groups				*p*
	K- (n = 6)	K+ (n = 7)	P1 (n = 4)	P2 (n = 4)	
Body Weight (g)					
Pre-	276.8 ± 23.20	266.1 ± 29.00	255.8 ± 19.29	291.8 ± 18.84	0.202*
Post-	294.7 ± 13.26[a]	280.1 ± 16.39[a]	281.5 ± 14.39[a]	307.3 ± 12.53[b]	0.034*
Δ	17.83 ± 26.32	14.00 ± 23.80	25.75 ± 22.57	15.50 ± 28.12	0.896*
p	0.157***	0.170***	0.106***	0.350***	
Fasting Blood Glucose (mg/dl)					
Pre-	96.98 ± 19.27[a]	155.18 ± 16.33[b]	196.00 ± 46.62[b]	181.75 ± 40.31[b]	0.000*
Post-	113.18 ± 8.59[a]	208.12 ± 103.24[b]	114.35 ± 6.18[a]	116.25 ± 5.12[a]	0.004**
Δ	16.20 ± 17.57[a]	52.94 ± 95.22[b]	-81.65 ± 50.04[c]	-65.50 ± 35.44[c]	0.003**
p	0.074***	0.398****	0.047***	0.034***	
Plasma Fibrinogen (mg/dl)					
Pre-	17.58 ± 4.23	51.57 ± 57.62	26.81 ± 12.99	16.49 ± 6.29	0.089**
Post-	20.4 ± 8.32	89.36 ± 202.04	24.94 ± 16.72	13.47 ± 5.29	0.559**
Δ	2.91 ± 8.44	37.79 ± 146.24	-1.86 ± 16.53	-3.02 ± 7.88	0.270**
p	0.437***	0.237****	0.836***	0.499***	

The value was expressed in Mean ± SD

*One-Way ANOVA Test, **Kruskal Wallis Test, ***Paired T-Test, ****Wilcoxon Test

[a,b,c,)] Different notations on the same line indicate significant differences

Characteristic of Plasma Fibrinogen

Table 2 indicates that there was an increase of plasma fibrinogen in control groups K- and K+ (Δ = 2.91 ± 8.44 mg/dl and 37.79 ± 146.24 mg/dl respectively). On the contrary, the plasma fibrinogen level in treatment groups P1 and P2 declined after intervention. The difference observed in the treatment group was -1.86 ± 16.53 mg/dl and -3.02 ± 7.88 mg/dl respectively. The highest decline was shown in delta group P2 (Δ = -3.02 ± 7.88 mg/dl), and there was no significant reduction of plasma fibrinogen level pre- and post-intervention ($p = 0.49$). The observed result indicates that the diabetic rats given vitamin D3-fortified kefir has stronger anticoagulant activity compared to unfortified kefir, which was shown in the decreased level of plasma fibrinogen after 35day intervention. Based on *One-Way ANOVA* test, there was no significant difference in plasma fibrinogen levels in all groups before and after an intervention ($p = 0.27$).

DISCUSSION

This study aims to determine the association between vitamin D3-fortified goat kefir on plasma fibrinogen level in diabetic rats. The results showed that vitamin D3-fortified goat kefir was statistically able to reduce plasma fibrinogen and blood glucose levels in diabetic rats. Diabetes was administered to the rats through the injection of STZ-NA. The control and treatment groups of diabetic rats developed hyperglycemia due to autoimmune mechanisms. This autoimmune mechanisms generated reactive oxygen species (ROS). ROS exceeding antioxidant levels induce oxidative stress. Oxidative stress reduces the immunes responses through activation of nuclear factor kappa beta (NfKB) and activating protein 1 (AP-1), lipid peroxidation, increased production of proinflammatory cytokine, and pancreatic B-cell damage. Imbalance between free radical production and antioxidant production leads to necrosis of pancreatic B-cells [28]. Furthermore, this condition will increase blood glucose level due to hyperglycaemia and affecting the inflammation status of the study sample. The inflammatory reaction that occurs in the early phase starts from neutrophils then macrophages enter the injured tissue. These cells will produce ROS, which has detrimental effects on surrounding tissues. Excessive production of ROS can cause tissue damage, haemostasis and interfere with the coagulation process. This condition makes the formation of plasma fibrin during the coagulation process takes a longer time to be dissolved and degraded. Increases in plasma fibrinogen, factor VII activity, and

plasminogen activator inhibitor (PAI) cause increased platelet aggregation and enhanced activation of plasma fibrinogen [29, 30].

In this study, K+, P1, and P2 group diabetics have plasma fibrinogen levels that are generally elevated in acute infections, are directly proportional to the hyperglycemic state in patients with diabetes mellitus, and are closely associated with the development of thrombosis [31]. Moreover, the results of the study using STZ-induced rats found that the longer duration of acclimatisation and the age of adult rats affecting fasting blood glucose and plasma fibrinogen levels [32]. The diabetic condition causes an increase in platelets due to thrombotic hyperactivity. Consequently, the activation of the prothrombotic coagulation factor increased, whereas fibrinolysis decreased. This circumstance explained the decrease in fasting blood glucose level in the current study sample, but the fibrinogen levels depletion was not significant. Another study found that the anti-diabetic effect of bacteria contained in kefir was able to significantly reduced blood glucose, HbA1c, and phosphate levels in patients with type-2 diabetes during the 10-week intervention. Similar studies found that administration of 2 ml/day of kefir causes an increase in inflammatory mediators on diabetic rats [25, 33]. Also, it has been reported that kefir may reduce glycemia and improves the balance of pro-inflammatory and anti-inflammatory cytokines [22, 34]. These findings were similar to the results of the current study, where the fasting blood glucose level of the intervention group decreased. In contrast, the plasma fibrinogen levels of the treatment groups indicate no significant differences before and after treatment. The occurrence of hyperglycaemia found in rats was affecting the haemostatic state and coagulation; thus, it stimulates excessive fibrinogen production [31]. The role of kefir as a probiotic and antioxidant administered in a diabetic situation directly functions as a fibrinogen polymerisation protector, and thereby it would accelerate the wound healing by preventing oxidative stress which can inhibit coagulation [21, 33, 35]. Several studies found the link of a high level of antioxidant activity in kefir to malondialdehyde (MDA) levels. MDA levels are used in measuring free radical activity. Kefir was reported to be able to reduce the MDA levels in diabetic rats [21].

Improvement in blood glucose and plasma fibrinogen levels in each treatment groups that received kefir showed that the probiotics contained in goat kefir have beneficial effects on type-2 diabetes treatment. The anti-diabetic effect from Lactobacillus and Bifidobacterial activity in kefir can reduce blood glucose levels in diabetic rats by stimulating glycogen formation in the liver from blood glucose and antioxidant status [36, 37]. The antioxidant status is directly influenced by oxidative stress, which occurs early in the development of diabetes. Diabetes condition causes insulin failure to stimulate glucose uptake by fat and muscle tissue, resulting in a high concentration of glucose in the blood. This condition results in oxidant products increment and causing damage to the defence triggered by antioxidant [37]. In the current study, the antioxidant status was not measured, but the decrease in fasting blood glucose was related to the antioxidant activity contained in kefir. The decline in blood glucose is also triggered by gut microbiota that produced insulinotropic polypeptides and glucagon, stimulating glucose uptake into muscles [22].

Meanwhile, the administration of vitamin D increases the sensitivity of insulin secretion when blood glucose levels are elevated. It modulates Peroxisome Proliferator-Activated - γ (PPAR-γ) signalling in glucose metabolism and inflammation process, also increase PPAR-γ expression during adipogenesis [33]. Vitamin D affects the ß-cells function and mass by increasing the proliferation of pancreatic ß-cells so that the ß-cells mass increases. The dose given to the P2 group was 600 IU (15 µg) of vitamin D3 based on the Recommended Dietary Allowances (RDAs) and The Indonesian Dietary Recommendation (AKG) 2019. A study found that the administration of high doses of vitamin D3 per-oral was able to delay the development of disturbances in high fasting blood glucose values in diabetic subjects, with the same dosage and concentration being carried out in experimental rats [38, 39]. The result of the study illustrated that vitamin D3 could increase calcification in blood vessels and stimulate proliferation of smooth-muscle cells in blood vessels. One factor that might distinguish the control group from the treatment group is the differences in the immune response of the rats. The rats that have low immunity have a higher chance of getting an infection, so they are susceptible to inflammation due to elevated fasting blood glucose even in condition without treatment [39]. This finding is in line with the development of diabetes mellitus in humans so that the dose of vitamin D3 is safe for use in experimental animals [38]. Based on the results of the study, vitamin D3-fortified kefir had a more significant effect on lowering fasting blood glucose levels in diabetic rats.

Moreover, there was a decrease in fibrinogen levels, accompanied by a decline in blood glucose levels in P1 and P2 groups. The low level of plasma fibrinogen in P1 and P2 groups indicated a reduction in inflammation and a decrease of disease severity in people with diabetes mellitus. Lactobacillus found in kefir activates innate immune receptors, named as toll-like receptors (TLRs) that are involved in activating pro-

inflammatory cytokines (TNF-a, IL-6). The IL-6 has a role in stimulating fibrinogen synthesis that occurs in the liver. The role of fibrinogen as a biomarker of inflammation is to carry out the coagulation process to maintain the haemostatic system [24, 40].

Fibrinogen levels was associated with glucose metabolism including fasting blood glucose and markers of diabetes mellitus type 2 [25]. A study found that a group of diabetic rats experienced a significant increase in fibrinogen levels due to streptozotocin injection [26]. The results of the fibrinogen measurement in the experimental rats recorded 0.93±0.46 mg/ml of fibrinogen. This increase lasted until the 96th hour, with fibrinogen level in 1.80±0.1 mg/ml. The study found that fibrinogen level increased as diabetes progressed [26]. The increase in fibrinogen level to the inflammation stage in diabetic rats ranged from 93 to 180 mg/dl. Based on these findings, all groups of diabetic rats had not yet reached the inflammatory stage. Based on a study observing the old category of type-2 diabetes towards fibrinogen, the highest duration of Type-2 Diabetes in patients was in the more than ten years category with a mean value of 690.9 mg/dl [41]. Also, in the results, the number of research samples that differed between the group was prone to bias. This is due to several samples of rats that died during the study.

The vitamin D3-fortified goat kefir affects the haemostatic state of diabetic rats, showing a lower fibrinogen result in P2 group. The intake of vitamin D-fortified goat kefir triggers high antioxidant activity in cell protection so that the inflammation does not occur [42]. The addition of vitamin D3 in goat kefir triggers the production of cytokines (IL-10.TNF-α, dan IL-6) and activates the nitric oxide (NO) release from the blood cells. The release of NO can be beneficial in inhibiting the atherogenic monocytes and LDL infiltration to the arterial walls. Also, the release of NO acts in inhibiting platelet aggregation and genes expression involved in atherogenesis [43]. To summarise, the role of goat kefir in this study is in line with the existing evidence from previous studies, which reported that goat kefir could prevent tissue damage in diabetic rats even though the results are not statistically significant.

CONCLUSIONS

Administration of 2 mL/200 g body weight/day of vitamin D3-fortified goat kefir (vitamin D3 fortification dose of 600 IU/day) lowers plasma fibrinogen among diabetic mice insignificantly.

ACKNOWLEDGMENT

We would like to express our gratitude to Research Development and Application of Diponegoro University, for the support given to this research through the Research Grant 2018. We would also like to thank all participating individuals for the encouragement and assistance throughout the completion of this study.

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e-ISSN : 2338-3119, p-ISSN: 1858-4942

Jurnal Gizi Indonesia
(The Indonesian Journal of Nutrition)
Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition)
Vol. 11, No. 2, June 2023 (85-94)
Submitted: 15 November 2022, Accepted: 01 May 2023
Online https://ejournal.undip.ac.id/index.php/jgi

ASSOCIATION OF EATING HABITS AND COOKING METHODS WITH BREAST TUMORS AMONG CHILDBEARING AGED URBAN WOMEN IN INDONESIA: A CROSS-SECTIONAL STUDY

Imaviana Cahyani[1], Khairizka Citra Palupi[1*], Adi Kurniawan[2], Leffiyanti Handi[3], Rathi Paramastri[4], Mertien Sa'pang[1]

ABSTRACT

Background*: breast tumors is the single most commonly detected benign or malignant tumors among women and has now become a global health burden.*

Objective: *This study aimed to determine the associations of eating habits and cooking methods with a breast tumor in childbearing-aged Indonesian urban women.*

Materials and Methods*: This was a cross-sectional study using a community survey of research of non-communicable disease 2016 database from the ministry of health of Indonesia. In total,28558 women, aged 25 – 49 years old were retrieved from the database. Eating habits and cooking methods were measured using a validated food frequency questionnaire. A forward logistic regression analysis was used to examine the association of eating habits and cooking methods with the risk of breast tumors.*

Results*: Higher education level was positively associated with the incidence of breast tumors (OR = 1.10, 95%CI: 1.01-1.20, p = 0.026). Seafood (OR = 0.88, 95% CI: 0.80-0.96, p = 0.006) and fast foods (OR = 1.10, 95% CI: 1.00-1.20, p = 0.049) were associated with the incidence of breast tumor among urban women. Roasted/smoked cooking method was positively associated with risk of breast tumor (OR = 1.27, 95%CI: 1.01 – 1.61, p = 0.043).*

Conclusion*: Our study is the first community-based study in Indonesia investigating the association of eating habits and cooking methods with the incidence of breast tumors among childbearing-aged urban women. High intake of seafood was associated with a lower risk of breast tumors, while fast foods and roasted/smoked cooking method belief to have a detrimental effect on a breast tumor. Prospective studies are needed to confirm the present study findings.*

Keywords *: eating habits, cooking methods, breast tumor, urban women*

BACKGROUND

A tumor is a mass of abnormal tissue. These are two types of breast tumors includes non-cancerous or benign and those are cancerous or malignant. Worldwide, the malignant breast tumors is the single most commonly detected malignant tumors among women and has now become a global health burden [1, 2]. In Indonesia, the incidence of malignant breast tumors continues to increase every year and represents the leading cause of malignant tumors mortality among women [2]. The incident rate of malignant breast tumors may differ by geographical location. Certain environmental and lifestyle factors including diet play a significant role to enhance malignant breast tumors [3]. Literature has shown that diet alone, can accounts for approximately 35% of all malignant tumor cases. Therefore, it is essential to recognize dietary habits including cooking methods for malignant breast tumors risk [4].

For the past years, a large number of studies have evaluated the association of particular foods with their composition including eating habits with the advancement of malignant breast tumors [5, 6]. According to the report released by World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) prospected an increase in malignant breast tumors risk among women eating higher-fat diets compared with those eating lower-fat diets [7]. Eating and cooking habits extremely vary among countries or regions. Soy food, which is mainly consumed in Asia has been found to have a low risk for malignant breast tumors among women [8], likewise, total dairy food consumption including milk also appears to be riskless

[1]Department of Nutrition and Public Health, Esa Unggul University, Indonesia
[2] Heidelberg Institute of Global Health, Faculty of Medicine, Heidelberg University, Germany
[3]Subang Hospital, Indonesia
[4]School of Nutrition and Health Sciences, College of Nutrition, Taipei Medical University, Taiwan
[*]**Correspondence** : E-mail: khairizka.citra@esaunggul.ac.id

e-ISSN : 2338-3119, p-ISSN: 1858-4942

[9]. In contrast, another prospective cohort study conducted in South Korea revealed that consuming high-cholesterol foods grilled meat, and inconsistent eating habits seems to be associated with an elevated risk of malignant breast tumors among adult women [10].

However, most previously published studies on the association of diet and malignant breast tumors were focus on single food items or nutrients. Investigating overall eating habits together with cooking methods might be more significant in determining breast tumors etiology rather than evaluating diets separately as a result of the complexity and combinations of food or nutrients among individuals [11, 12]. Following healthy eating habits such as fruits, vegetables, fish, olive oil and whole grains is likely to be advantageous to reduce malignant breast tumors among middle-aged women [13]. A study has reported that moderate consumption of pan-fried/bread-coated fried white meat and well-done or stewed red meat have a minimal risk for malignant breast tumors. Moreover, the total intake of processed/cured meat was considered for having a strong association with triple-negative tumors [14]. Preventive dietary measures to deteriorate malignant breast tumors risk could also include the use of olive/liquid oils for cooking and quitting the use of mayonnaise as salad dressing [15]. Since limited studies using a large sample size particularly among Indonesian urban women investigating these issues, therefore in this study, we aimed to determine the associations of eating habits and cooking methods with a breast tumor on childbearing-age in Indonesian women.

MATERIALS AND METHODS

Study population

This cross-sectional study was used a database from a community survey of research of non-communicable disease in 2016. This survey was conducted by the Department of research and development Ministry of Health of Republic Indonesia. The major aim of this survey was to obtain the prevalence rate of women with positive breast tumors in urban areas in Indonesia. The cross-sectional study was conducted on August – September 2016 in selected 76 districts/cities using cluster random sampling from 34 provinces. The target sample was 70.000 urban women. The inclusion criteria were women age 24-65 years and willing to participate in research and sign informed consent. The exclusion criteria were women with serious physical illness, very severe communication disorders, pregnant and breastfeeding women less than 6 months. Data was collected through interviews and the interviews were conducted at the participant's house by trained enumerators. The enumerators would ask a series of general and personal questions including education status and occupation. In total, 39.188 women were recruited by Ministry of Health of Republic Indonesia. After excluded those who were ≥ 50 years old (n=10258) and not having breast tumors examination (n=372), a total of 28.558 women were used for the analysis. This study was approved by Esa Unggul University's ethical review board (0180-20.175/DPKEKEP FINAL-EA/UEU/VII/2020).

Assessment of breast tumors

In this study, the examination of breast tumors used the clinical breast examination method. A certified medical doctor or midwife carried out the clinical breast examination (inspection and palpation) at the primary healthcare clinics. They assessed breast position (asymmetric or not), the skin of the breast (normal, reddish, swollen, wet wounds or pulling), and the areola of the breast (normal, retracted, wet wounds or abnormal fluid appears). Moreover, the palpation examination was carried out to check the lump (number, size, quadrant location, consistency, mobility, and tenderness) and the presence of enlarged lymph nodes at the armpits. The subjects were categorized as positive if the clinical breast examination show the lump and the presence of enlarged lymph nodes at the armpits and inversely.

Assessment of eating habits, cooking methods, and other covariates

A food frequency questionnaire (FFQ) was used to assess participants eating habits and cooking methods in the last month. In the FFQ, 106 food items (Supplementary file 1) were categorized into twelve food groups (meats, eggs, innards organs, processed meats, seafood, milk and dairy products, fast foods, beans or legumes, light-colored vegetables, dark leafy vegetables, fruits, and oils). We also assessed the habit of alcohol drinking. Meanwhile, seven types of cooking methods, namely roasted/smoked, fried, grilled, boiled, pan-fried/sautéing, steamed, and raw/fresh were also retrieved from the FFQ. Each food item and cooking method had seven response scores (1 to 7) ranged from never, 1x/month, 2-3x/month, 1-2x/week, 3-4x/week, 5-6x/week, and daily. Meats and dark-leafy vegetables consist of seven food items. Processed meats, seafood, and fast foods consist of four food items. Milk and dairy products and beans or legumes consist of nine food items. While eggs, innards organs, light-colored vegetables, fruits, and oils consist of 3, 5, 18, 30, and 6 food

items respectively. The minimum and maximum consumption scores for meats, eggs, innards organs, processed meats, seafood, milk and dairy products, fast foods, beans or legumes, light-colored vegetables, dark leafy vegetables, fruits, and oils are 7-49, 3-21, 5-35, 4-28, 4-28, 9-63, 4-28, 9-63, 18-126, 7-49, 30-210, 6-42, respectively. Eating habits for each food group were categorized as low (below the median/2-quintile) and high (above the median/2-quintile). Moreover, the cooking methods were categorized as a 'regular' method if they used roasted/smoked, fried, boiled, pan-fried/sautéing, steamed, and raw/fresh cooking methods ≥ 3-4x/week, and grilled cooking method ≥ 2-3x/month, and 'infrequent' if otherwise [10]. Other covariates such as education status were dichotomized as low (below high school) and high (above high school). While working status was categorized as no (not working or still studying) and yes (engaged in professional working).

Data analysis

Statistical analysis was performed using STATA version 13 (STATA Corp LLC, Texas, USA). A chi-squared test was used to compare the categorical variables among the characteristics of women with breast tumors incidence. A general linear model was used to compare the eating habits score of each food group according to breast tumors incidence. Meanwhile, an adjusted (age, education, and working status) and forward selection of logistic regression was performed to determine the most dominant variables associated with breast tumors among urban women. We used forward logistic regression tests with a predictive model by selecting each independent variable. First, all independent variables that produced *p-value* < 0.25 were selected for further analysis. Second, variables that have met the previous selection criteria will be adjusted one by one (variables with p-value > 0.05 will be removed gradually) until there were only variables with p < 0.05.

RESULTS

From a total of 28558 women, 8.3% (n = 2376) of those had a breast tumor. Characteristics of urban women across breast tumors status are shown in Table 1.

Table 1. Characteristics of study participants according to breast tumors status

All (n = 28558)		Breast tumors		p [a]
		Negative (n = 26182)	Positive (n = 2376)	
Age, years	38.4 ± 6.6	38.4 ± 6.6	38.6 ± 6.6	
Education level				0.028*
Low	13788 (48.3)	12692 (48.5)	1096 (46.1)	
High	14770 (51.7)	13490 (51.5)	1280 (53.9)	
Working status				0.05*
No	18222 (63.8)	16750 (64.0)	1472 (62.0)	
Yes	10336 (36.2)	9432 (36.0)	904 (38.0)	
Alcohol drinking				0.368
No	27695 (97.0)	25398 (97.0)	2297 (96.7)	
Yes	863 (3.0)	784 (3.0)	79 (3.3)	
Food consumption score				
Meats	11.5 ± 3.3	11.5 ± 3.2	11.7 ± 3.4	0.006*
Eggs	7.6 ± 3.2	7.6 ± 3.2	7.7 ± 3.3	0.242
Innards organs	5.8 ± 1.5	5.8 ± 1.5	5.9 ± 1.6	0.096
Processed meats	5.8 ± 2.3	5.8 ± 2.3	5.9 ± 2.3	0.473
Seafood	10.2 ± 3.7	10.2 ± 3.7	10.0 ± 3.8	0.011*
Milk and dairy products	11.1 ± 3.1	11.1 ± 3.1	11.3 ± 3.3	0.015*
Beans or legumes	19.0 ± 4.9	19.0 ± 4.9	19.0 ± 4.9	0.554
Light colored vegetables	35.5 ± 12.6	35.5 ± 12.6	35.7 ± 12.7	0.609
Dark-leafy vegetables	15.3 ± 4.4	15.3 ± 4.4	15.4 ± 4.5	0.861
Fruits	44.6 ± 12.3	44.6 ± 12.3	44.5 ± 12.3	0.877
Oils	13.4 ± 2.8	13.4 ± 2.8	13.5 ± 3.0	0.500
	4.8 ± 1.6	47 ± 1.6	4.8 ± 1.7	0.010*

	All (n = 28558)	Breast tumors		P [a]
		Negative (n = 26182)	Positive (n = 2376)	
Fast foods				
Cooking method [b]				
Roasted/smoked				0.035*
Infrequent	27756 (97.2)	25463 (97.2)	2293 (96.5)	
Regular	802 (2.8)	719 (2.8)	83 (3.5)	
Deep-fried				0.045*
Infrequent	2449 (8.6)	2219 (8.5)	230 (9.7)	
Regular	26109 (91.4)	23963 (91.5)	2146 (90.3)	
Grilled				0.066
Infrequent	25384 (88.9)	23299 (89.0)	2085 (87.8)	
Regular	3174 (11.1)	2883 (11.0)	291 (12.2)	
Boiled				0.998
Infrequent	13943 (48.8)	12783 (48.8)	1160 (48.8)	
Regular	14615 (51.2)	13399 (51.2)	1216 (51.2)	
Pan-fried/Sautéing				0.589
Infrequent	11846 (41.5)	10848 (41.4)	998 (42.0)	
Regular	16712 (58.5)	15334 (58.6)	1378 (58.0)	
Steamed				0.093
Infrequent	27005 (94.6)	24776 (94.6)	2229 (93.8)	
Regular	1553 (5.4)	1406 (5.4)	147 (6.2)	
Raw/fresh				0.564
Infrequent	16254 (56.9)	14915 (57.0)	1339 (56.4)	
Regular	12304 (43.1)	11267 (43.0)	1037 (43.64)	

Data are presented as number (percentage) for categorical variables and mean ± standard deviation(SD) for continuous variables.

[a] P-value was analyzed by chi-square for categorical variables and a general linear model for continuous variables.

[b] 'Regular' method if they used roasted/smoked, fried, boiled, pan-fried/sautéing, steamed, and raw/fresh cooking methods ≥ 3-4x/week, and grilled cooking method ≥ 2-3x/month, and 'infrequent'if otherwise

* significant (p-value < 0.05)

Positive breast tumors women had higher education levels (53.9%), higher consumption score of meats (11.7 ± 3.4 vs. 11.5 ± 3.2; p = 0.006), milk and dairy products (11.3 ± 3.3 vs. 11.1 ± 3.1; p = 0.015), and fast food (4.8 ± 1.7 vs. 4.7 ± 1.6; p = 0.010) but lower intake of seafood (10.0 ± 3.8 vs. 10.2 ± 3.7; p = 0.011). Additionally, positive breast tumors women used fried cooking method regularly (90.3%, p = 0.045).

Logistic regression analysis revealed that seafood and fast food were significantly associated with the incidence of breast tumors (Table 2).

Table 2. Logistic regression of eating habits and incidence of breast tumors

	Adjusted logistic regression		Forward logistic regression	
	OR (95% CI)	P	OR (95% CI)	P
Meats				
Low	1.00		1.00	
High	1.02 (0.94 – 1.11)	0.605	1.00 (0.91 – 1.10)	0.969
Eggs				
Low	1.00		1.00	

	Adjusted logistic regression		Forward logistic regression	
	OR (95% CI)	**P**	**OR (95% CI)**	**P**
High	1.08 (0.99 – 1.18)	0.072	1.09 (0.99 – 1.20)	0.069
Innards organs				
Low	1.00		1.00	
High	1.04 (0.95 – 1.13)	0.444	1.03 (0.93 – 1.14)	0.572
Processed meats				
Low	1.00		1.00	
High	0.98 (0.90 – 1.07)	0.705	0.94 (0.85 – 1.04)	0.234
Seafood				
Low	1.00		1.00	
High	0.91 (0.84 – 0.99)	0.028	0.88 (0.80 – 0.96)	0.006*
Milk and dairy products				
Low	1.00		1.00	
High	1.07 (0.99 – 1.17)	0.105	1.06 (0.97 – 1.16)	0.167
Beans or legumes				
Low	1.00		1.00	
High	1.02 (0.94 – 1.11)	0.658	1.01 (0.92 – 1.11)	0.835
Light-colored vegetables				
Low	1.00		1.00	
High	0.99 (0.92 – 1.09)	0.973	0.98 (0.88 – 1.10)	0.760
Dark-leafy vegetables				
Low	1.00		1.00	
High	1.02 (0.93 – 1.11)	0.693	1.02 (0.92 – 1.12)	0.720
Fruits				
Low	1.00		1.00	
High	1.01 (0.93 – 1.10)	0.871	0.99 (0.89 – 1.10)	0.911
Oils				
Low	1.00		1.00	
High	0.98 (0.90 – 1.07)	0.677	0.98 (0.90 – 1.08)	0.732
Fast foods				
Low	1.00		1.00	
High	1.11 (1.02 – 1.22)	0.018	1.10 (1.00 – 1.20)	0.049*

[a] adjusted by age, education level, and working status

* significant (p-value < 0.05)

Low: < median/2-quintile scores of 11.0 (meats), 7.0 (eggs), 5.0 (inn,ards organs), 5.0 (processed meats), 10.0 (seafood), 9.0 (milk and dairy products), 18.0 (beans or legumes), 33.0 (light coloredvegetables), 15.0 (dark-leafy vegetables), 41.0 (fruits), 12.0 (oils), and 4.0 (fast foods); high if otherwise.

Adjusted and forward logistic regression showed that seafood negatively associated with breast tumors (OR = 0.91, 95% CI: 0.84 – 0.99, p = 0.028 and OR = 0.88, 95% CI: 0.85 – 1.04, p = 0.006 respectively). In contrast, fast foods were associated with increased 10% risk of breast tumors (95% CI: 1.00 – 1.20, p = 0.049). Furthermore, only roasted/smoked cooking method was positively associated with the incidence of breast tumors (OR = 1.27, 95% CI: 1.01 – 1.61, p = 0.043) (Table 3).

Table 3. Logistic regression of cooking methods and incidence of breast tumors

	Adjusted logistic regression[a]		Forward logistic regression	
	OR (95% CI)	**P**	**OR (95% CI)**	**P**
Roasted/smoked				
Infrequent	1.00		1.00	
Regular	1.28 (1.01 – 1.61)	0.04	1.27 (1.01 – 1.61)	0.043*
Deep-fried				
Infrequent	1.00		1.00	
Regular	0.86 (0.74 – 0.99)	0.038	0.87 (0.75 – 1.01)	0.066
Grilled				
Infrequent	1.00		1.00	
Regular	1.12 (0.99 – 1.28)	0.079	1.10 (0.96 – 1.26)	0.168
Boiled				
Infrequent	1.00		1.00	
Regular	0.99 (0.91 – 1.08)	0.894	0.99 (0.91 – 1.09)	0.911
Pan-fried/Sautéing				
Infrequent	1.00		1.00	
Regular	0.98 (0.90 – 1.06)	0.586	0.98 (0.90 – 1.07)	0.637
Steamed				
Infrequent	1.00		1.00	
Regular	1.16 (0.97 – 1.38)	0.100	1.13 (0.94 – 1.35)	0.186
Raw/fresh				
Infrequent	1.00		1.00	
Regular	1.01 (0.93 – 1.10)	0.821	1.03 (0.94 – 1.12)	0.550

[a] adjusted by age, education level, and working status

"Infrequent" was defined as ≤ 1-2 times/week (roasted/smoked, fried, boiled, pan-fried/sautéing,steamed, raw/fresh) and ≤ 2-3 times/month (grilled) while "regular" if otherwise.

DISCUSSION

In this study, our evaluation showed that the incidence of breast tumors in Indonesia was significantly associated with higher education levels and fast foods as well as roasted/smoked cooking methods among childbearing-aged urban women. Whereas, total seafood consumption was substantially associated with a low risk of breast tumors incidence. The evidence relating to individual education level to risk for breast tumors is still unclear. Nonetheless, education differences in cancer prevalence have long been observed [16]. However, it is not well known, if such a relationship will continue to exist in Indonesia following adjustment for individual risk factors of breast tumors. The findings of this present study are similar to previous publications of elevated risk of breast tumors among women with higher education levels [17, 18]. A prospective cohort study conducted in the United State has shown that women with the least education level had a lower risk of invasive malignant breast tumors [16]. In another study, the relationship between education level and malignant tumors risk becomes completely insignificant after multivariate adjustment for unhealthy lifestyles [19]. Perhaps, such analysis might give a plausible description about the connection between education and cancer for public health concern. Inconsistent results have also been observed in several studies conducted to investigate the role of education level in breast tumors survival. Among the studies, one reported no relationship found between education and breast tumors [20], while others revealed a high survival rate among women with lower [21] or higher [22] education level.

Eating habits have been identified to involve both in the development and prevention of breast tumors [23] but the evidence is yet to be cleared [24]. Since breast tumors is a hormone-related malignancy, the diet may enhance its consequences through the hormonal level, anti-oxidation as well as growth factors [25, 26]. From the literature review, we noticed an increased risk of breast tumor-associated with high consumption of fast foods, which similarly concurred with our study [27]. Fast foods are typically meat and sweet diets with a large quantity of sugar and fat, prepared as baked goods, burgers, and deep-fried foods like chips, french fries, and chicken pieces [7]. Furthermore, fast foods have been linked to a diet prepared with limited essential nutrients and frequent intake of it may lead to overconsumption of energy [28]. High energy consumption may trigger the release of essential hormonal factors such as estrogen, insulin-like growth factor (IGF)-1, and sex hormone-binding globulin, which all play a significant role in the proliferation of malignant tumor cells [29]. A group of researchers from Poland has reported that consuming fast food daily (100 gr) is threefold likely to increase the risk of malignant breast tumors. In their study, they further discovered that refined meat including burgers, sausage, and pizza containing sodium nitrate, is transformed into nitrosamine, which is considered carcinogen [30].

Cooking methods vary among regions or countries; therefore, differences in the incidence rate of breast tumors may occur. The type of cooking methods applied for preparing diet may also have an impact on malignant tumors cell growth. In the recent study, our findings indicated that prepared meals by roasting or smoking are associated with an elevated risk of breast tumors. The roasted or smoked cooking method involved the use of high temperatures to prepare food. During our literature review, no study specifically reported that consuming high-temperature cooked food, for example eating grilled/barbecued and smoked meat, is associated with primary prevention of breast tumors, it is however been reported that women to reduce consuming high-temperature cooked meat due to the formation of carcinogenic compounds [31]. A study conducted among US females showed that grilled/barbecued and smoked meat consumption are the main origin of polycyclic aromatic hydrocarbons (PAHs) [32] and have been regarded to increase the proportion of breast tumors [33, 34]. PAHs together with another carcinogenic mutagen such as heterocyclic amines (HCAs) are found highly in meat cooked at high temperatures [35]. HCAs are formed when muscle meat which consists of amino acids and creatinine reacts at high temperatures [36]. Meanwhile, PAHs are formed at the surface of the meat when it cooked directly on an open flame, resulting in pyrolysis of the fat. Imperfectly combusted meat also leads to carbon and hydrogen from fat react with hot coals and produce smoke [35].

Seafood has been identified associated with breast tumors. Increasing evidence has emerged showing an increased risk of breast tumors linked with the intake of seafood (like fresh-water fish and marine fish), which is not in support of other literature conducted distinctively [37- 39]. Our results are, however, similar to other publications, in which malignant tumors risk is reduced with high seafood consumption [40]. Seafood such as fish (oily fish or fish oils) is regarded as the major source of n-3 polyunsaturated fatty acids (n-3 PUFA), which can effectively deteriorate the risk of malignant breast tumors among women [41, 42]. In addition, fish oil or a diet rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may also inhibit metastases development and further suppress tumors progression [43-45]. However, the epidemiologic evidence showing the relationship between seafood and breast tumors risk is limited to our understanding.

Our study has certain strengths: it is the first study to examine the association of eating habits and cooking methods among childbearing Indonesian urban women. Second, the sample size was selected from 34 provinces in Indonesia, thus it may represent all areas of the Indonesian archipelago. However, several limitations should also be mentioned. First, the cross-sectional study design limits us to identify the causality of the results. Second, potential misclassification bias of self-reported FFQ may occur and the questionnaire included only frequency information, not portion size. Therefore, the intake amount for each participant could not be calculated. Finally, the adjustment for confounding variables was modest in our study. Additional residual confounding variables such as energy and protein intake, genetic or family history, and nutritional status may affect the present findings.

CONCLUSIONS

In summary, higher education levels, consumption of fast foods, and roasted/smoked cooking methods were identified as major risk factors associated with the incidence of breast tumors among childbearing-aged urban women living in Indonesia. In contrast, only seafood was discovered having reduced risk for breast tumors. The current study appears to provide the possible high- and low-risk factors associated with breast tumors incidence in Indonesia. Therefore, it is substantial to establish awareness and disseminate information about good eating habits and cooking methods to minimize the risks of breast tumors. The findings of this study suggest that changes in eating habits and cooking methods might impact how breast tumors generate.

However, the relationship between eating habits and cooking methods in breast tumors is complicated, more studies might be required for clear evidence.

ACKNOWLEDGMENT

The authors thank the department of research and development, Ministry of Health of Republic Indonesia for collecting and providing their database available for this study.

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Supplementary Table 1. Food groups and food items list

Food groups	Food items
Meats	Chicken, chicken skin, duck, beef, pork, lamb, and buffalo meat
Eggs	Chicken egg, duck egg, egg yolk
Innards organs	Chicken liver, brain, chicken blood, cow liver, cow intestine
Processed meats	Corned beef, meat ball, sausage, cow tripe
Seafood	Shrimp, fish, salted fish, dried fish
Milk and dairy products	Liquid skim milk, powder skim milk, yogurt, cheese, lamb milk, cow milk, sweetened condensed milk, buffalo milk, full cream milk
Fast food	Fried chicken, burger/hotdog, pizza, French fries
Beans or legumes	Green bean, soy bean, red bean, peanut, black-eyed pea, oncom, tofu, tempeh, soy milk
Light vegetables	Green bean, luffa acutangula, baby corn, cabbage, cucumber, cauliflower, yardlong bean, winged bean, chayote, white radish, young jackfruit, bitter melon, young papaya, bamboo shoot, mung bean sprout, eggplant, tomato, carrot
Dark-leafy vegetables	Spinach, broccoli, choy sum, limnocharis flava, water spinach, Gnetum gnemon leaves, bok choy
Fruits	Avocado, grapes, red apple, green apple, star fruit, cantaloupe, langsat, durian, watery rose apple, guava, orange, ambarella, Japanese persimmon, lychee, mango, mangosteen, passion fruit, melon, jackfruit, pineapple, pear, papaya, banana, rambutan, sapodilla, salak, watermelon, soursop, sugar apple, strawberry
Oils	Coconut, margarine, butter, coconut oil, palms oil, coconut milk

e-ISSN : 2338-3119, p-ISSN: 1858-4942

Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition)
Vol. 11, No. 2, June 2023 (95-109)
Submitted: 27 November 2022, Accepted: 15 February 2023
Online https://ejournal.undip.ac.id/index.php/jgi

SMALL DENSE LOW-DENSITY LIPOPROTEIN CHOLESTEROL AND CENTRAL OBESITY ASSOCIATED WITH DIABETES MELLITUS AMONG INDONESIAN ADULTS

Yeni Rohmaeni[1*], Hardinsyah Hardinsyah[1], Ikeu Tanziha[1]

ABSTRACT

Background: Small dense low-density lipoprotein (sdLDL-C) is an atherogenic lipoprotein. Increased sdLDL-C concentration was hypothesized to be associated with obesity and diabetes mellitus (DM). **Objectives**: The study aimed to determine the association between sdLDL-C, central obesity, and DM among Indonesian adults, controlled by personal and clinical parameters.

Materials and Methods: This study used secondary data from Basic Health Research 2013 of the Ministry of Health, which applied a cross-sectional study design. For this purpose, 30,548 subjects aged 19-79 were analyzed. The sdLDL-C was performed by using Sampson Formula derived from conventional lipid panels. As investigated by Sampson, the formula referred to cLDL-C (calculated LDL-C) and ElbLDL-C (estimated large buoyant LDL-C).

Results: There was a positive association between sdLDL-C and central obesity (OR: 3.94; 95% CI: 3.13-3.89), as well as sdLDL-C and DM status (OR: 1.98; 95% CI; 1.43-2.75) after adjusting the personal and clinical parameters.

Conclusion: This study demonstrated that the increment of sdLDL-C level and central obesity affected DM status in Indonesian adults. It implies that the sdLDL-C was a potential biomarker to assess the risk of DM.

Keywords: Central obesity, Diabetes mellitus, Hypertension, Indonesian adult, sdLDL-C

BACKGROUND

The prevalence of diabetes mellitus has risen worldwide over the last decade, and it is projected to increase further to 700 million by the year 2045. According to the national data, Indonesia has shown an increasing trend of diabetes mellitus. The number of 15 years and more with DM increased from 2007 to 2018, according to medical providers diagnosed [1], and it seems Indonesia has a higher prevalence than the Asia Pacific region [2]. DM is a complex disease, and dyslipidemia via insulin resistance is a critical causal factor in the development of many acute complications, including stroke, coronary artery disease (CAD), and renal destruction [3].

The numerous factors of DM are smoking, low physical activity, dietary pattern, age, gender, and dyslipidemia [4–10]. In addition, some studies considered the association of DM with education level, wealth [11], and place of living [12, 13]. Since diabetic dyslipidemia is highly prevalent in subjects with type 2 DM [14], the study of 140.557 subjects in Thailand showed that more than half of the subjects with T2DM had abnormalities in LDL-C, triglyceride (TG), and HDL-C [3]. The primary feature was slightly increased LDL-C, TG, and decreased HDL-C [15, 16]. Otherwise, LDL-C has a range in sizes and densities [17]. There is the scientific judgment that sdLDL-C as pattern B was more atherogenic since it has been characterized by small dense (< 25.5 nm) and lower affinity [18], they have a greater risk of endothelial penetration and causes of arterial stiffening [19, 20]. The current opinion states that insulin resistance in offspring was the leading cause of CVD events via vascular stiffening [21, 22]

The laboratory analysis method varies; the homogeneous assay and ultracentrifugation are commonly used [17, 18]. However, many researchers have developed the equation to calculate sdLDL-C derived from conventionally measured lipid panels [23, 24] according to the need for more technologies in the clinical setting, cost and time effectiveness being a consideration. The previous study on the association between sdLDL-C and CVD in Indonesia was investigated in children of 5-9 years [25], adults [22], DM subjects [26], and obese subjects [22] in a small sample size. Although sdLDL-C has been investigated in obese subjects, no studies have evaluated central obesity and DM status among Indonesian adults. Indonesia has enormous data on lipid profiles and potentially be used for calculating sdLDL-C concentration, with this data can mitigate a potential risk factor of elevated sdLDL-C, central obesity, and DM status in each characteristic. The current

[1] Department of Community Nutrition, Faculty of Human Ecology, IPB University, Indonesia
[*]**Correspondence** : E-mail: ikeutanziha@gmail.com

e-ISSN : 2338-3119, p-ISSN: 1858-4942

study aimed to determine the association between sdLDL-C, central obesity, and DM status by controlling personal and clinical parameters.

MATERIALS AND METHODS

Design and Sampling

This study analyzed the secondary data using a cross-sectional study of Basic Health Research 2013 (Riset Kesehatan Dasar 2013) collected by the National Institute of Health Research Development (NIHRD), Ministry of Health, Indonesia. The protocol and study method of Basic Health Research has been published in detail elsewhere [1]. The study subject was selected by multistage stratified sampling (fig.1). The final subject was drawn from a 1000-block census of province estimation which was nationally representative. The potentially eligible subject was 34,007, and the advanced analysis was completed from February to July 2022.

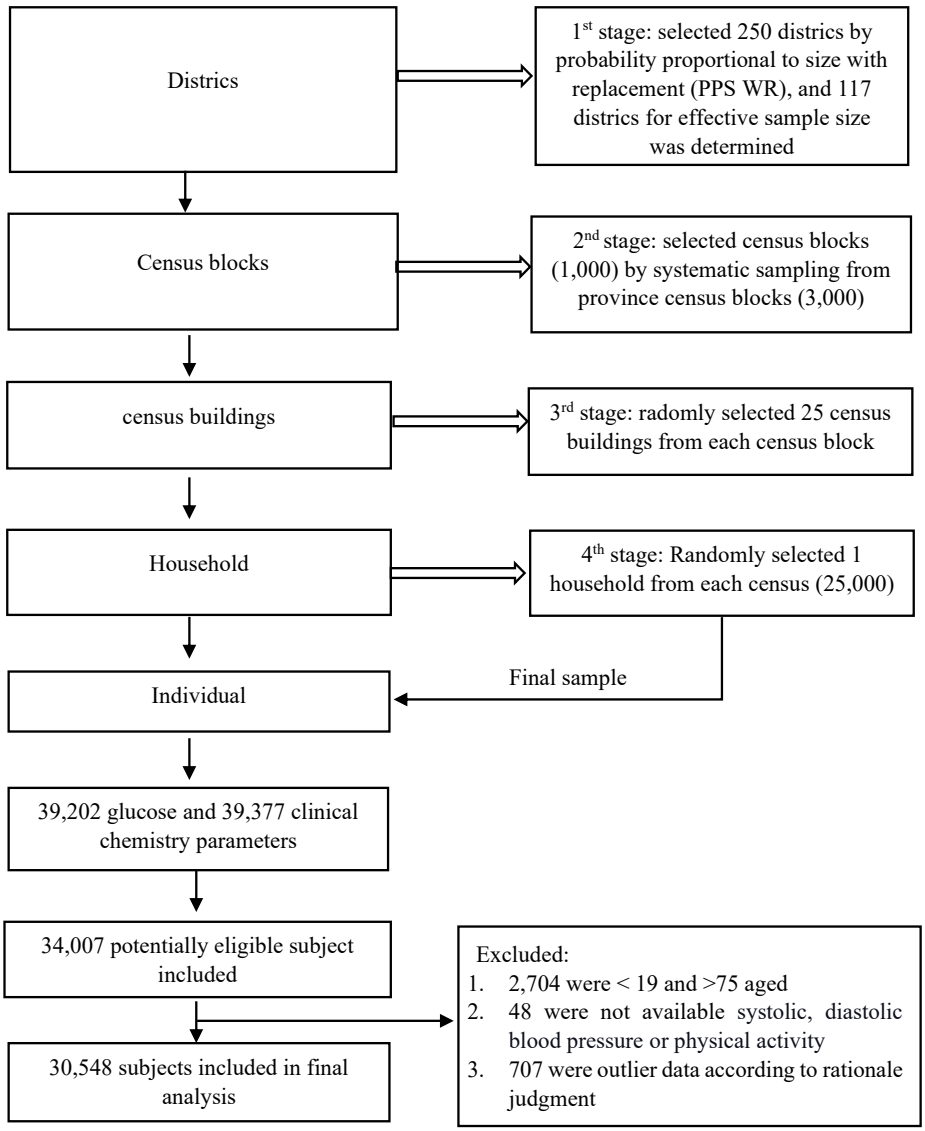

Fig 1. Flow diagram of selection study subjects. Modified from The Ministry of Health[1].

Data Processing

The subjects were Indonesian, aged 19-79 years, and well-trained laboratory analysts took subjects assessed in clinical biochemistry. Missing data on blood pressure and physical activity, and TG concentration was 800 mg/dL above, excluded from the study. The number of subjects was 34,007, and 30,548 were included in the analysis after applying the exclusion criteria. Each parameter of plasma glucose was carried out to examine the correlational analysis of personal and clinical parameters with plasma glucose. The number of subjects with glucose parameters was 2.201, 23.635, and 6.913 for 2 hours, fasting and random plasma glucose, respectively.

Sociodemographic Parameters

Direct interviews with a questionnaire were used to collect sociodemographic parameters, including age, gender, place of living, education attainment, occupation, and economic status. This study used a newly redefined age group and the possible link between disease in human lifespan by Giefman et al. [2]; the age study subjects were stratified into four categories;19-33 years, 34-48 years, 49-64 years, and ≥ 65 years. Education attainment is classified into three categories: 'primary' (less than senior high school), 'secondary' (senior high school), and 'college' (diploma or above). Five categories of occupation are used; farmer/fisher/laborer, professional worker, self-employed, other, and unemployment. Economic status was assessed by Principal Component Analysis (PCA), resulting in quintiles from lowest to highest. The correlational polychoric was used to generate the PCA matrix. Only the variable with more than 0.3 correlational value and more than 0.5 proportion explained can be used as a predictor economic status variable [1, 3]. There were 12 selection variables, including water supply, cooking fuel type, toilet usage, toilet type, disposal habits, lighting type, and ownership of the motorcycle, TV, water heater, 12 kg gas cylinder, refrigerator, and car.

Smoking Habit, Physical Activity, and Dietary Fruits and Vegetables

Smoking status was classified as non-smoker, former smoker, and smoker. A modified GPAQ (Global Physical Activity questionnaire) was used to assess vigorous and moderate physical activity and sedentary behavior. Physical activity level was defined by calculating METs per minute for each dimension by multiplying 4.0 and 8.0 METs for vigorous and moderate over a week period [2]. Calculated physical activity levels on Mets/minute/week were then classified as sedentary, low, moderate, and active. Sedentary time was defined as not having moderate or intense physical activity on any day of the previous week. In comparison, high physical activity was defined as taking a score ≥3000 METs-minute/week. Moderate physical activity was taking a score of 2999-600 METs-minute/week, and low physical activity failed to meet any criteria above. Dietary fruits and vegetables were collected by a simple questionnaire that asked consumption frequency per week and the serving size per day, thus were categorized into: never, <3 portions per day, 3-4 per day, and ≥5 per day.

Clinical Parameters

The sdLDL-C was defined following the Sampson equation: elbLDL-C: 1.43xLDL-C – (0.14x(ln(TG)xLDLC))- 8,99, and the sdLDL-C: LDL C–elbLDL-C that refers to current calculated LDL-C (cLDL-C) equation which proposed by Sampson [2]. A new equation of cLDL-C seems more accurate and possibly be used in patients with low LDL-C levels and hypertriglyceridemia (TG levels, ≤800 mg/dL) than the previous equation [3]. The body mass index based on the Quetelet index (kg/m^2) was defined into three criteria [4]; normal (≥25.0), overweight (25.1-27.00), and obesity (≥27.1), and central obesity was defined as a waist circumference of ≥ 90 and ≥80 cm [5], for man and women respectively.

Lipid parameters were classified according to the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel [6]. The abnormal values were defined following lipid levels; ≥200 mg/dl (TC), 100 mg/dl (LDL-C), and 150 gr/dl (TG), while HDL-C was <40 mg/ dl and <50 mg/dl for men and women respectively. High levels of creatinine were referred to in the previous study (≥1.2 and ≥1.1 mg/dl for men and women, respectively) [7]. The blood pressure was defined according to global hypertension practice guidelines (systolic≥ 140 mmHg or diastolic ≥90 mmHg) [8]. DM was defined according to plasma glucose measurement referring to ADA classification. The cut-off was fasting plasma glucose ≥126 mg/dL or 2 hours (postprandial) plasma glucose ≥200 mg/dl or random plasma glucose ≥200 mg/dL with the classic symptom [9] or refer to the diagnosis of medical providers.

Statistical Analysis

Data were analyzed using SPSS 25 version, summary statistic results of subject characteristics stratified by quintile of sdLDL-C. Data were expressed as a percentage for categorical variables and mean±standard deviation for continuous variables. Cut-off sdLDL-C were defined as Q1 (≤ 24.86 mg/dl), Q2 (24.87-31.06 mg/dl), Q3 (30.07-37.74 mg/dl), Q4 (37.75-46.11 mg/dl), and Q5 (≥ 46.12 mg/dl). Quintile 1 was chosen as a reference and considered the lowest-risk group for the outcome. Kruskal-Wallis tests were performed to analyze variables across quintiles for continuous variables. Single binary logistic regression analyses were used to attain the association between each variable and DM with 95% CI and multinomial logistic regression for each variable and sdLDL-C across quintiles. The strength of the association was expressed by adjusted odds ratios (aOR) and 95% CI. $p<0.05$ was recognized as statistically significant.

RESULTS

Characteristics of the Study Subjects According to Quintile sdLDL-C

The baseline personal and clinical characteristics of the study subjects are summarized in Table 1. The average age was 42.59 ± 15.78 years old. More than half of the subjects resided in rural areas, there was a similar proportion of men and women, nearly three-quarters had low education (73,0; 95% CI: 71,7-74,3), and more than three-quarters (75.2; 95% CI: 71.2-73.8) were classified as active. The percentage of participants who consumed the recommended amounts of fruits and vegetables was relatively low. Although LDL-C and 2h-PG results tended to be higher than normal, the overall mean clinical parameters tended to classify within normal values.

Table 1. Baseline characteristic of the study subjects (n:30,548)

Variabel	% (95% CI)[a]
Age (years)	42.59±15.78
Gender	
Men	49.9 (48.7-51.1)
Women	50.1 (48.9-51.3)
Place of living	
Urban	43.7 (43.1-44.4)
Rural	56.3 (55.6-56.9)
Education attainment	
Primary	73.0 (71.7-74.3)
Secondary	22.0 (21.0-23.1)
Collage	4.9 (4.4-5.5)
Occupation	
Farmer/fisher/laborer	33.5 (32.2-34.7)
Professional worker	10.9 (10.2-11.8)
Self-employed	13.7 (13.0-14.5)
Others	3,9 (3.5-4.3)
Unemployment	38.0 (37.0-39.0)
Economic status	
Lowest	13.8 (12.6-15.2)
Low	20.5 (19.3-21.8)
Middle	24.5 (23.3-25.8)
High	24.2 (23.0-25.5)
Highest	16.8 (15.6-18.2)
Smoking Habit	
Never	61.7 (61.0-62.5)
Former	5.0 (4.7-5.4)
Current	33.2 (32.5-34.0
Physical activity	
Sedentary	7.4 (6.6-8.2)
Low	3.3 (2.9-3.6)
Moderate	16.9 (16.0-17.8)
Active	75.2 (71.2-73.8)
Dietary Fruits and Vegetable	
Never	0.9 (0.7-1.1)
< 3 (portion/day)	80.0 (78.8-81.2)
3-4 (portion/day)	16.1 (15.5-17.6)
≥ 5 (portion/day)	2.6 (2.3-3.0)
BMI (kg/m^2)	23.1±4.2
Waist circumference (cm)	78.5±11.0
TC (mg/dl)	188.1±39.8
HDL-C (mg/dL)	41.7±15.0
LDL-C (mg/dL)	126.10±35.27
TG (mg/dl)	123.4±74.8
sdLDL-C (mg/dL)	35.9±13.0
2h-FG (n=2.201)	142.3±51.7
FPG (n=23.635)	104.4±30.2

Variabel	% (95% CI)[a]
RPG (n=6.913)	114.7±41.3
Creatinine (mg/dL)	0.81±0.25
Sistolic (mmHg)	128.2±21.3
Diastolic (mmHg)	82.4±11.9

Data illustrated the weighted percentages and 95% CI for the categorical variable and mean±SD for the continuous variable.
BMI: Body Mass Index; TC: Total Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; TG: Triglycerides; 2h-FG: 2-Hour Plasma Glucose; FPG: Fasting Plasma Glucose; RDP: Random Plasma Glucose; sdLDL-C: Small Dense LDL-C According to Sampson Equation.

The distribution of personal and clinical risk factors of DM according to the quintile sdLDL-C are summarized in Table 2. The proportion of quintile 5 sdLDL-C (≥ 46.12) was higher in 49-64 aged and higher in men than women and urban than rural populations. The study also showed that quintile 5 was highly prevalent in college education level, self-employed, and subjects with the highest quintile economic status. In addition, the proportion of sdLDL-C levels increased gradually across the quintile from the subject who smoked and never consumed fruits and vegetables. In contrast, sdLDL-C levels decreased gradually in the physically active subjects.

Table 2. Percentages of Subjects for Each Personal Risk Factor of DM Status According to sdLDL-C Quintile

Variable	Quintile sdLDL-C (95% CI)				
	Q1	Q 2	Q3	Q 4	Q5
Age (years)					
19-33	31.7 (30.3-	24.7 (23.4-	19.5 (18.4-	14.3 (13.3-	
34-48	33.1)	26.0)	20.7)	15.4)	9.8 (8.9-10.7)
49-64	16.5 (15.6-	20.3 (19.4-	20.9 (20.0-	21.4 (20.5-	20.8 (19.8-
≥ 65	17.6)	21.2)	21.9)	22.4)	21.8)
	11.0 (10.1-	15.3 (14.3-	20.1 (18.8-	23.7 (22.5-	29.9 (28.5-
	12.1)	16.4)	21.3)	25.0)	31.3)
	13.4 (11.7-	19.4 (17.5-	21.8 (19.9-	21.6 (19.6-	23.9 (21.7-
	15.2)	21.4)	23.8)	23.7)	26.2)
Gender					
Men	16.6 (15.7-	20.2 (19.3-	21.0 (20.0-	21.4 (20.4-	20.9 (19.9-
Women	17.6)	21.1)	21.9)	22.3)	21.9)
	22.9 (21.9-	20.9 (20.1-	19.8 (19.1-	18.2 (17.4-	18.2 (17.4-
	23.9)	21.7)	20.6)	18.9)	19.0)
Place of living					
Urban	19.9 (19.6-	19.6 (18.7-	19.6 (18.8-	19.3 (18.3-	21.5 (20.4-
Rural	19.6)	20.6)	20.5)	20.3)	22.7)
	20.4 (21.5-	21.5 (20.7-	21.0 (20.2-	19.8 (19.1-	17.2 (16.3-
	21.0)	22.4)	21.9)	20.6)	18.1)
Education attainment					
Primary	19.5 (18.6-	20.7 (20.0-	20.8 (20.2-	20.2 (19.5-	18.7 (17.9-
Secondary	20.5)	21.4)	21.6)	20.9)	19.5)
Collage	22.7 (21.2-	20.9 (19.5-	18.8 (17.5-	17.9 (16.6-	19.7 (18.4-
	24.3)	22.4)	20.0)	19.2)	21.2)
	18.3 (15.9-	17.3 (14.7-	19.6 (17.2-	17.9 (15.7-	26.8 (23.8-
	21.1)	20.3)	22.2)	20.4)	30.1)
Occupation	18.5 (17.4-	22.0 (21.0-	21.7 (20.7-	20.6 (19.6-	17.1 (16.1-
Farmer/fisher/laborer	19.7)	23.1)	22.7)	21.7)	18.1)
Professional worker	19.9 (18.0-	18.2 (16.5-	19.4 (17.5-	20.3 (18.4-	22.2 (20.4-
Self-employed	22.0)	20.1)	21.4)	22.4)	24.0)
Others	16.4 (14.9-	18.5 (17.0-	20.4 (18.9-	20.5 (18.9-	24.2 (22.6-
Unemployment	18.1)	20.1)	21.9)	22.1)	26.0)
	21.6 (18.8-	18.1 (15.5-	19.6 (16.5-	21.4 (18.6-	19.3 (16.8-
	24.7)	21.0)	23.0)	24.6)	22.1)
	22.9 (21.7-	21.0 (20.1-	19.5 (18.5-	17.9 (16.9-	18.8 (17.8-
	24.1)	22.0)	20.5)	18.8)	19.8)
Economic status					
Lowest	22.2 (20.3-24.2)	22.0 (20.6-23.5)	21.0 (19.6-22.4)	19.7 (18.1-21.4)	15.1 (13.7-16.6)
Low	21.5 (19.9-23.1)	21.7 (20.5-22.9)	22.1 (20.9-23.4)	18.6 (17.4-19.9)	16.1 (14.9-17.3)
Middle	20.8 (19.4-22.3)	21.8 (20.6-23.1)	20.2 (19.1-21.4)	19.0 (17.9-20.2)	18.1 (17.0-19.2)

Variable	Quintile sdLDL-C (95% CI)				
	Q1	Q 2	Q3	Q 4	Q5
High	19.4 (18.0-20.9)	19.3 (18.2-20.4)	20.0 (18.8-21.3)	20.1 (18.8-21.4)	21.2 (19.9-22.6)
Highest	17.0 (15.7-18.4)	18.2 (16.8-19.7)	18.2 (16.9-19.5)	20.6 (19.2-22.0)	26.0 (24.3-27.8)
Smoking Habit					
Never	22.6 (21.6-23.5)	21.0 (20.2-21.7)	19.8 (19.1-20.6)	21.7 (19.8-19.1)	20.6 (18.4-17.7)
Former	14.8 (12.4-17.6)	19.5 (17.1-22.1)	20.3 (17.8-23.0)	22.1 (20.3-17.8)	23.0 (20.9-18.5)
Current	16.5 (15.5-17.6)	20.1 (19.0-21.2)	21.3 (20.2-22.4)	21.2 (21.3-20.2)	22.4 (21.5-20.5)
Physical activity					
Sedentary	18.8 (16.1-21.9)	20.5 (18.1-23.1)	18.4 (16.2-20.8)	20.5 (18.2-23.1)	21.8 (19.2-24.7)
Low	20.3 (17.1-24.0)	17.3 (14.6-20.5)	18.3 (15.2-21.8)	19.2 (16.4-22.4)	24.8 (21.5-28.5)
Moderate	20.5 (19.0-22.2)	19.3 (17.8-20.8)	19.9 (18.5-21.5)	19.1 (17.8-20.5)	21.2 (19.7-22.7)
Active	20.2 (19.4-21.1)	21.1 (20.4-21.8)	20.7 (20.0-21.4)	19.6 (18.8-20.4)	18.4 (17.7-19.2)
Dietary Fruits and Vegetable					
Never	18.6 (13.0-25.7)	18.7 (13.1-26.0)	19.9 (14.4-26.9)	18.1 (12.7-25.2)	24.7 (19.6-30.5)
< 3 (portion/day)	20.4 (19.5-21.3)	20.5 (19.9-21.2)	20.2 (19.5-20.9)	19.7 (18.9-20.4)	19.2 (18.4-19.9)
3-4 (portion/day)	19.5 (18.0-21.1)	21.2 (19.8-22.8)	20.8 (19.4-22.2)	19.0 (17.6-20.5)	19.4 (17.9-21.0)
≥ 5 (portion/day)	16.9 (13.7-20.6)	18.8 (15.3-22.9)	21.3 (18.0-25.0)	20.3 (17.1-23.8)	22.8 (19.0-27.0)

Data ilustrated the weighted percentages and 95% CI. sdLDL-C value defined as Q1 (≤ 24.86 mg/dl), Q2 (24.87-31.06 mg/dl), Q3 (30.07-37.74 mg/dl), Q4 (37.75-46.11 mg/dl) and Q5 (≥ 46.12 mg/dl).

The Kruskal-Wallis test was used to assess the mean in different clinical parameters according to quintile sdLDL-C are shown in Table 3. As expected, the mean BMI, waist circumference, TC, LDL-C, TG, and 2h-PG, FPG, RP, creatinine, and blood pressure were significantly increased, while HDL-C declined across the quintiles.

Table 3. Mean of Clinical Risk Factors of DM Status According to sdLDL-C Quintiles

Variable	Quintile sdLDL-C (mean±SD)					*p-value*
	Q1 n: 6,105	Q 2 n: 6,109	Q3 n: 6,105	Q 4 n: 6,113	Q5 n: 6,116	
BMI (kg/m^2)	21.6±3.6	22.3±3.9	23.1±4.0	23.7±4.2	24.7±4.2	**<0.001**
Waist circumference (cm)	74.2±9.3	76.1±10.1	78.0±10.6	80.2±11.0	83.6±11.2	**<0.001**
TC	147.2±26.1	168.7±22.1	184.6±22.1	201.8±21.9	238.1±32.1	**<0.001**
HDL-C	52.6±14.8	50.7±11.8	49.0±11.6	46.9±11.3	44.3±10.7	**<0.001**
LDL-C	89.7±20.7	109.0±18.4	123.6±19.8	138.4±20.9	169.1±31.1	**<0.001**
TG	64.3±28.8	89.5±33.8	112.3±41.8	143.4±58.0	207.2±93.1	**<0.001**
2h-FG (n=2.201)	132.4±38.7	135.4±43.6	140.3±45.7	142.9±51.0	160.9±.69.8	**<0.001**
FPG (n=23.635)	97.8±16.1	100.0±19.2	102.4±23.3	105.3±30.5	116.6±47.8	**<0.001**
RPG (n=6.913)	107.1±31.2	110.0±29.3	111.2±30.3	114.6±39.0	128.3±61.0	**<0.001**
Creatinine (mg/dl)	0.75±0.23	0.79±0.24	0.81±0.23	0.83±0.24	0.86±0.30	**<0.001**
Sistolic (mmHg)	121.6±18.5	125.1±20.1	128.0±21.1	130.8±21.4	135.4±22.7	**<0.001**
Diastolic (mmHg)	79.1±10.7	80.7±11.20	82.1±11.5	83.8±12.0	86.1±12.5	**<0.001**

BMI: Body Mass Index; TC: Total Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; TG: Triglycerides; 2h-FG: 2-Hour Plasma Glucose; FPG: Fasting Plasma Glucose; RDP: Random Plasma Glucose; sdLDL-C: Small Dense LDL-C According to Sampson Equation.
Kruskal-Wallis test for continuous values
The bold number was statistically significant.

Correlational Analysis Between Personal and Clinical Parameters with Plasma Glucose

The coefficient correlation (r) is shown in Table 4. Spearman analysis showed that sdLDL-C was independently correlated with plasma glucose parameters, and the highest r value was found in the FPG parameter (r=0.211). It was also found that BMI, waist circumference, TC, LDL-C, TG, creatinine, systolic, and diastolic blood pressure were statistically positively correlated with plasma glucose. In contrast to METs-minute/week, dietary fruits and vegetables and HDL-C tended to be negatively associated with plasma glucose.

Table 4. The r and p Values of Personal and Clinical Parameters with Plasma Glucose Correlation

Variables	2h-PG (n=2,201)		FPG (n= 23,635)		RPG (n=6,913)	
	r	p-value	r	p-value	r	p-value
Age (years)	0.199	**<0.001**	0.254	**<0.001**	0.264	**<0.001**
Mets-minute/week	-0.077	**<0.001**	0.001	0.908	-0.008	0.525

e-ISSN : 2338-3119, p-ISSN: 1858-4942

Variables	2h-PG (n=2,201)		FPG (n= 23,635)		RPG (n=6,913)	
	r	p-value	r	p-value	r	p-value
Sedentary time	0.022	**<0.001**	-0.006	0.353	0.007	0.544
Dietary Fruits and Vegetable	0.017	**0.012**	-0.002	0.718	-0.002	0.842
BMI (kg/m^2)	0.103	**<0.001**	0.058	**<0.001**	0.080	**<0.001**
Waist circumference (cm)	0.124	**<0.001**	0.100	**<0.001**	0.113	**<0.001**
TC	0.145	**<0.001**	0.144	**<0.001**	0.115	**<0.001**
HDL-C	-0.018	**0.008**	-0.085	**<0.001**	-0.103	**<0.001**
LDL-C	0.140	**<0.001**	0.141	**<0.001**	0.105	**<0.001**
TG	0.116	**<0.001**	0.213	**<0.001**	0.188	**<0.001**
Creatinine (mg/dl)	-0.083	**<0.001**	0.043	**<0.001**	0.175	**<0.001**
Systolic (mmHg)	0.179	**<0.001**	0.201	**<0.001**	0.034	**0.004**
Diastolic (mmHg)	0.150	**<0.001**	0.112	**<0.001**	0.204	**<0.001**
sdLDL-C	0.153	**<0.001**	0.211	**<0.001**	0.112	**<0.001**

BMI: Body Mass Index; TC: Total Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; TG: Triglycerides; 2h-FG: 2-Hour Plasma Glucose; FPG: Fasting Plasma Glucose; RDP: Random Plasma Glucose; sdLDL-C: Small Dense LDL-C According to Sampson Equation
The bold number was statistically significant

Correlational Analysis Between sdLDL-C, Central Obesity, and DM Status

The OR and 95% CI of personal and clinical parameters among the sdLDL-C quintiles are shown in Table 5. Clustering of age demonstrated a positive association between sdLDL-C and age. In comparison, in Q5 vs. Q1, the 49-64 years group owned the highest risk of sdLDL-C (OR: 8.89; 95% CI: 7.69-10.28), while gender consideration demonstrated that women were found to have about 35% lower risk increasing of sdLDL-C level than man (OR: 0.65; 95% CI: 0.59-0.71). Similarly, place of living showed that subjects living in rural areas found about a 23% decrease in sdLDL-C (OR: 0.77; 95% CI: 0.68-0.88). Given education attainment, subjects who completed a diploma or above tended to increase sdLDL-C (OR: 1.59; 95% CI: 1.27-1.99), as seen in the professional and self-employed occupation group (OR: 1.22; 95% CI: 1.03-1.44; OR:1.62; 95% CI:39-1.89, respectively). The study also showed that the highest economic status group increased the 2.29-time risk of elevated sdLDL-C (OR: 2.29; 95% CI: 1.86-2.76).

The study identified smoking habits, physical activity, and dietary fruits and vegetables as risk factors related to sdLDL-C. The study showed that former and current smokers have an increased risk of elevated sdLDL-C (OR: 1.95; 95% CI: 1.54-2.46; OR: 1.51: 95% CI: 1.37-1.66, respectively) contrary to physical activity reduced by 22 % (OR: 0.78; 95% CI: 0.62-0.98) the risk of elevated sdLDL-C. The results showed no association between sdLDL-C and dietary fruits and vegetables. A cross quintile of sdLDL-C obesity subjects was founded to increase the risk of elevated sdLDL-C as seen in central obesity (OR: 5.09; 95% CI: 4.40-5.90; OR: 3.49; 95% CI: 3.13-3.89, respectively). The study also found the increase of creatinine and blood pressure strongly associated with elevated sdLDL-C (OR: 4.46: 95% CI: 3.40-5.87; OR: 3.31; 95% CI: 2.96-3.69, respectively).

Table 5. The OR and 95% CI Values of Personal and Clinical Parameters According to sdLDL-C Quintiles

Variable	Quintile sdLDL-C (mean±SD)				
	Q1	Q 2	Q3	Q 4	Q5
Age (years)					
19-33	ref	ref	ref	ref	ref
34-48		**1.56 (1.41-1.74)**	**2.04 (1.83-2.28)**	**2.89 (2.59-3.23)**	**4.00 (3.51-4.56)**
49-64		**1.82 (1.59-2.08)**	**2.99 (2.60-3.44)**	**4.81 (4.18-5.53)**	**8.89 (7.69-10.28)**
≥ 65		**1.94 (1.60-2.58)**	**2.56 (2.11-3.11)**	**3.69 (3.06-4.45)**	**5.75 (4.65-7.10)**
Gender					
Men	ref	ref	ref	ref	ref
Women		**0.77 (0.71-0.85)**	**0.69 (0.62-0.76)**	**0.64 (0.58-0.70)**	**0.65 (0.59-0.71)**
Place of living					
Urban	ref	ref	ref	ref	ref
Rural		1.05 (0.95-1.16)	1.05 (0.94-1.17)	0.97 (0.87-1.09)	**0.77 (0.68-0.88)**
Education attainment					
Primary		ref	ref	ref	ref
Secondary	ref	**0.85 (0.75-0.97)**	**0.74 (0.66-0.84)**	**0.87 (0.69-0.88)**	0.90 (0.79-1.03)
Collage		0.90 (0.72-1.14)	1.01 (0.82-1.24)	0.97 (0.78-1.20)	**1.59 (1.27-1.99)**

Variable	Quintile sdLDL-C (mean±SD)				
	Q1	Q 2	Q3	Q 4	Q5
Occupation					
Farmer/fisher/laborer	ref	ref	ref	ref	ref
Professional worker		**0.75 (0.64-0.88)**	0.85 (0.71-1.01)	0.96 (0.80-1.15)	**1.22 (1.03-1.44)**
Self-employed		0.95 (0.81-1.11)	1.08 (0.93-1.26)	1.14 (0.98-1.32)	**1.62 (1.39-1.89)**
Others		**0.73 (0.58-0.92)**	**0.73 (0.56-0.95)**	0.93 (0.73-1.18)	0.99 (0.73-1.18)
Unemployment		**0.77 (0.69-0.85)**	**0.74 (0.66-0.82)**	**0.72 (0.64-0.81)**	0.91 (0.81-1.02)
Economic status					
Lowest	ref	ref	ref	ref	ref
Low		1.01 (0.88-1.16)	1.05 (0.89-1.23)	0.97 (0.81-1.17)	1.07 (0.89-1.27)
Middle		1.01 (0.89-1.27)	0.99 (0.85-1.17)	1.01 (0.84-1.22)	**1.24 (1.04-1.49)**
High		0.93 (0.81-1.08)	1.07 (0.90-1.27)	1.16 (0.97-1.39)	**1.56 (1.29-1.89)**
Highest		1.04 (0.89-1.22)	1.16 (0.89-1.22)	**1.36 (1.13-1.69)**	**2.29 (1.86-2.76)**
Smoking habit					
Never	ref	ref	ref	ref	ref
Former		**1.33 (1.04-1.71)**	**1.51 (1.19-1.91)**	**1.58 (1.26-1.97)**	**1.95 (1.54-2.46)**
Current		**1.29 (1.17-1.42)**	**1.47 (1.33-1.63)**	**1.55 (1.40-1.72)**	**1.51 (1.37-1.66)**
Physical activity					
Sedentary	ref	ref	ref	ref	**ref**
Low		0.87 (0.63-1.21)	0.88 (0.62-1.25)	0.96 (0.70-1.32)	1.08 (0.78-1.50)
Moderate		0.93 (0.73-1.17)	1.02 (0.79-1.32)	0.91 (0.72-1.14)	0.89 (0.69-1.16)
Active		1.00 (0.81-1.23)	1.06 (0.85-1.32)	0.93 (0.76-1.13)	**0.78 (0.62-0.98)**
Dietary Fruits and Vegetable					
never	ref	ref	ref	ref	ref
< 3 (portion/day)		0.91 (0.53-1.55)	0.97 (0.57-1.65)	0.99(0.55-1.80)	0.68(0.44-1.04)
3-4 (portion/day)		0.97 (0.56-1.67)	1.04 (0.61-1.78)	1.00(0.55-1.81)	0.72(0.46-1.12)
≥ 5 (portion/day)		1.06 (0.57-1.96)	1.14 (0.63-2.06)	1.21(0.64-2.29)	0.96(0.57-1.62)
General obesity					
Normal	ref	ref	ref	ref	ref
Overweight		1.26 (1.06-1.50)	1.79 (1.53-2.09)	2.06 (1.76-2.42)	3.16 (2.72-3.66)
Obesity		1.65 (1.42-1.93)	2.23 (1.91-2.60)	3.16 (2.74-3.65)	5.09 (4.40-5.90)
Central Obesity					
Normal	ref	ref	ref	ref	ref
Obesity		1.39 (1.24-1.56)	1.68 (1.51-1.88)	2.29 (2.05-2.55)	3.49 (3.13-3.89)
Creatinine Level					
Normal	ref	ref	ref	ref	ref
High		1.55 (1.16-2.06)	1.88 (1.40-2.53)	2.63 (2.01-3.45)	4.46 (3.40-5.87)
Blood pressure					
Normal	ref	ref	ref	ref	ref
Hypertension		1.37 (1.22-1.53)	1.72 (1.54-1.93)	2.33 (2.08-2.60)	3.31 (2.96-3.69)

*The OR illustrated the weight analysis
The bold number was statistically significant
ref was reference values

The association between sdLDL-C, central obesity, and DM status is shown in table 5. Increasing age was associated with an increased risk of DM status. In crude analysis, the study found that sex and DM were statistically significant (OR: 1.37; 95% CI: 1.25-1.50) for women, and DM was not significant comparing rural vs. urban. The study found that professional workers had a lower risk of DM status (OR: 0.77; 95% CI: 0.63-0.94). In contrast, other and unemployed groups had a higher risk of DM status than Farmer/fisher/laborer (OR: 1.41; 95% CI: 1.11-1.78; OR: 1.25; 95% CI: 1.11-1.41, respectively). We did not find the risk of economic status and DM status.

The analysis showed a higher risk of DM status for a former smoker (OR: 1.24; 95% CI: 1.03-1.50). In contrast, current smokers had a lower risk of DM status (OR. 0.68: 95% CI. 0.62-0.76) vs. the non-smoker group. This study showed the inverse association between physical activity and DM. Moderate and vigorous activity decreased 22% and 34% risk of DM status (OR 0.78: 95% CI. 0.64-0.95; OR. 0.66: 95% CI. 0.55-0.80, respectively) compared to the sedentary group, while there was not statistically significant difference between dietary fruits and vegetables with DM status.

These analyses demonstrated a significant association of sdLDL-C across quintile, as well as obesity and central obesity with DM status (OR: 3.92: 95% CI; 3.34-4.36; OR 1.96; 95% CI; 1.72-2.21; OR: 2.02; 95% CI: 1.83-2.22. respectively). In addition, the increment of TC, LDL-C, and TG levels increased the risk of DM 2.12, 1.89, and 2.02 times, respectively. Similar trends were also found in creatinine and hypertension. Subjects with a high level of creatinine and hypertension were more likely than the normal group (OR: 1.91; 95% CI: 1.58-2.31; OR: 2.46; 95% CI: 2.23-2.71. respectively). However, this analysis did not find any significant association with HDL-C.

Table 6. Crude and Adjusted OR 95% CI Values of Personal and Clinical Parameters with DM Status Correlation

Variabel	OR (95% CI)[a]	
	Crude	adjusted
Age (years)		
19-33	ref	ref
34-48	**2.94 (2.50-3.45)**	**2,40 (2,02-2,86)**
49-64	**5.93 (5.02-7.00)**	**4,25 (3.52-5.13)**
≥ 65	**7.42 (6.13-8.98)**	**5.45 (4.40-6.76)**
Gender		
Men	ref	ref
Women	**1.37 (1.25-1.50)**	1.03 (0.86-1.24)
Place of living		
Urban	ref	
Rural	1.08 (0.96-1.22)	-
Education attainment		
Primary	ref	ref
Secondary	**0.60 (0.53-0.69)**	**0.83 (0.72-0.96)**
Collage	**0.75 (0.58-0.96)**	0.78 (0.60-1.02)
Occupation		
Farmer/fisher/laborer	ref	ref
Professional worker	0.77 (0.63-0.94)	0.88 (0.75-1.02)
Self-employed	1.06 (0.90-1.25)	0.88 (0.71-1.11)
Others	**1.41 (1.11-1.78)**	0.90 (0.76-1.07)
Unemployment	**1.25 (1.11-1.41)**	1.27 (0.99-1.61)
Economic status		
Lowest	ref	
Low	0.84 (0.71-0.99)	-
Middle	0.86 (0.73-1.02)	
High	0.96 (0.80-1.14)	
Highest	0.98 (0.81-1.18)	
Smoking habit		
Never	ref	ref
Former	**1.24 (1.03-1.50)**	1.00 (0.78-1.27)
Current	**0.68 (0.62-0.76)**	**0.78 (0.66-0.93)**
Physical activity		
Sedentary	**ref**	
Low	1.01 (0.75-1.35)	1.10 (0.82-1.48)
Moderate	**0.78 (0.64-0.95)**	0.84 (0.69-1.03)
Active	**0.66 (0.55-0.80)**	0.83 (0.69-1.00)
Active		
Dietary Fruits and Vegetable (portion/day)		
Never	ref	
< 3	0.74 (0.48-1.13)	-
3-4	0.83 (0.54-1.28)	
≥ 5	0.79 (0.48-1.31)	
General obesity		
Normal	ref	
Overweight	**1.47 (1.27-1.69)**	1.07 (0.91-1.26)
Obesity	**1.96 (1.74-2.21**)	**1.28 (1.10-1.50)**
Central Obesity	ref	ref
Normal	**2.02 (1.83-2.22)**	**1.34 (1.17-1.53)**

Variabel	OR (95% CI)[a]	
	Crude	adjusted
Obesity		
High TC		
Normal	ref	ref
High	**2.12 (1.93-2.33)**	0.97 (0.84-1.12)
Low HDL-C		
Normal	ref	
Low	0.96 (0.87-1.05)	-
High LDL-C		
Normal	ref	ref
High	**1.89 (1.67-2.14)**	0.97 (0.81-1.16)
High TG		
Normal	ref	ref
High	**2.02 (1.84-2.23)**	**1.21 (1.05-1.40)**
Quintile sdLDL-C		
Q1	ref	ref
Q2	**1.26 (1.05-1.51)**	1.05 (0.85-1.29)
Q3	**1.69 (1.41-2.02)**	1.28 (1.01-1.62)
Q4	**2.02 (1.69-2.42)**	1.26 (0.96-1.66)
Q5	**3.92 (3.32-4.63)**	**1.98 (1.43-2.75)**
Creatinine Level		
Normal	ref	ref
High	**1.91 (1.58-2.31)**	**1.24 (1.02-1.51)**
Blood pressure		
Normal	ref	ref
Hypertension	**2.46 (2.23-2.71)**	**1.36 (1.22-1.51)**

TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglycerides; 2h-FG: 2-hour plasma glucose; sdLDL-C: Small dense low-density lipoprotein cholesterol according to Sampson equation
The OR illustrated the weight analysis
The bold number was statistically significant; ref was the reference group.

Table 6 also showed the adjusted OR and 95% CI value, quintile 5 vs. quintile 1 of sdLDL-C and DM. The result shows that sdLDL-C and central obesity remained positively associated with DM status. The subject in the highest quintile had double the odds of DM (aOR; 1,98; 95% CI: 1.43-2.75) than the subject with the lowest quintile, while the subject with central obesity was observed at 1.34 times the odds of DM (95% CI: 1.17-1.53) than normal subjects. The highest quintile of sdLDL-C and central obesity was independently associated with DM.

DISCUSSION

The study showed a positive association between sdLDL-C and obesity and sdLDL-C and DM status. The result showed increasing mean plasma glucose across quintiles. It is similar to the previous study reported by Sriswasdi et al. [2] and Izumida et al. [3]. sdLDL-C was a subclass of type LDL-C. There are two types of LDL-C: pattern A [(large buoyant (lbLDL-C)] and pattern B [(small dense LDL-C (sdLDL-C)] [4]. sdLDL-C was considered an atherogenic subclass of LDL-C since its characteristic has a small size and more sustain in the artery wall [4] as the leading cause of vascular stiffening [5]. A crucial aspect led to cardiovascular diseases (CVD) is diabetic dyslipidemia or atherogenic dyslipidemia, which is signed by elevated sdLDL-C, elevated TG, and decreased HDL-C [6, 7]. Numerous conditions stimulated the raising of sdLDL-C, but the primary factor correlated with lipid profile abnormalities is obesity [8], especially central obesity [9].

A present study found that general obesity and central obesity were strongly correlated with sdLDL-C. The results showed that sdLDL-C level increased gradually with the value of BMI and waist circumference ($p < 0.001$). Furthermore, a smaller study in Thailand showed that 58% of the obese subject had LDL-C peak density (gr/ml) ≥1.033, which is considered sdDLDL-C [10], and the increment of sdLDL-C was also found in people with metabolic syndrome [9].

sdLDL-C is a lipoprotein fraction derived from very low-density lipoprotein (VLDL). Substrates for lipoprotein lipase-mediated triglyceride [11]. There are two subclasses of VLDL: VLDL 1 and VLDL 2. In generic conditions, VLDL 1 is lower than VLDL 2. VLDL 1 is TG-rich content as an essential substrate for hepatic lipase. Moreover, hydrolyzed TG becomes a small and high-density LDL-C [4, 11]. Commonly, people

with insulin resistance secreted VLDL 1 higher than usual [11]. The original mechanism is promoted by CETP (cholesteryl esters transfer protein), which transfers plasma TG from VLDL1 to LDL-C. At the same time, CETP transfers CE (cholesterol esters) from LDL to VLDL1 and develops TG-rich LDL-C. Then, sdLDL-C is formed from TG-rich LDL-C as a precursor [4, 6, 11].

This study analyzed the sociodemographic parameters associated with sdLDL-C and DM. The result showed that age, sex, place of living, occupation, education attainment, and economic status were strongly associated with sdLDL-C. Compared with the previous study that showed no association between sdLDL-C and age [12]. The variation in results is likely due to the respondent's varying age characteristics. sdLDL-C level in men tends to be higher than in women. It may be caused by the likelihood of smoking being higher among men than women. Current male smokers had higher sdLDL-C concentrations than women (34.6 vs. 25.0 mg/dl, respectively) [13]. The occupation except for farmer/fisher/laborer and the highest quintile of economic status tends to be higher in the sdLDL-C level, and obesity may be related to this issue. Individuals living in urban areas had a higher sdLDL-C level: urbanization may trigger lipid disorders [14]. It was proven by Mohan et al. that sdLDL-C was significantly higher in urban diabetic subjects [15]. This phenomenon indicated that the risk of DM is indistinguishable in urban and rural areas. Similarly, a study in Malaysia showed no significant association between place of living and DM [16]. We assumed dietary patterns in rural areas related to socioeconomic [17], high consumption of sugar [18], and access issues of public healthcare may play a role in this condition [19].

Behavior risk factors include smoking habits, physical activity, and dietary fruits and vegetables. All of them, however, were crucial factors in elevated sdLDL-C. Our study showed that a former smoker had a higher sdLDL-C. Nicotine may promote a rising VLDL via secreted hormones. cortisol and catecholamine. This condition, however, may trigger the increase of fatty acid and TG-rich lipoprotein, a precursor of sdLDL-C [13]. The study showed a positive association between former smokers and the risk of DM status, confirmed by the previous study in China [20, 21]. It may be associated with an overall cumulative exposure to smoking before quitting. Possibility judgment is the 'weight cycling' phenomenon that occurs in weight gain and the increase in waist circumference, influencing the development of insulin resistance [22]. The previous study showed that BMI was associated with insulin resistance [23]. On the contrary, there was a negative association between DM and current smokers: the more significant energy expenditure and suppressed appetite were possible mechanisms that directly impact nicotine on energy balance [22, 24].

Among subjects, a group meeting recommendation for physical activity was negatively associated with sdLDL-C and DM status. The experimental study showed that moderate physical activity changed the mean LDL-C particle. Consequently, lipid profile and oxidative stress status benefit from increasing the clearance of circulating sdLDL-C [25]. The present study showed no significant association between dietary fruits and vegetables and DM, in contrast to a large study showing that ≈5 servings of fruits and vegetables were associated with reduced mortality of chronic diseases [26]. Although there was no association between dietary fruits and vegetables in multivariate analysis, the negative association was demonstrated in correlational analysis.

In addition, the generic risk factors of DM are hypertension and increased creatinine level. Our finding of an association between hypertension and creatinine level showed consistency with the previous study [12, 27] a significant association between sdLDL-C, hypertension, and creatinine level. Table 3 shows mean systolic, diastolic, and creatinine levels across quintiles. It suggested that sdLDL-C has an intercorrelation role with other clinical parameters in developing DM.

This study determined sdLDL-C, central obesity, and DM. To the best of our knowledge, this is the first study to investigate the relationship between sdLDL-C, central obesity, and DM on a large, nationwide scale. Furthermore, the numerous limitations of our study must be noticed. First, our cross-sectional study cannot investigate the causality between sdLDL-C, central obesity, and DM. Second, we only used the formula to find sdLDL-C concentration, but it is more effective, cheaper, and less time-consuming than laboratory measurement.

Further studies in experimental laboratories may be needed. Furthermore, although the sample size adequately represents the Indonesian population, the formula may be efficiently used to estimate sdLDL-C at the population level. The result may need to be generalizable to other populations with an advanced study design that can answer the causality of sdLDL-C and DM.

CONCLUSION

In conclusion, our findings demonstrated an association between sdLDL-C, central obesity, and the development of DM. Comprehensive prevention in lifestyle modification, such as dietary patterns, and

e-ISSN : 2338-3119, p-ISSN: 1858-4942

physical activity will be advantageous. The future multiethnic investigation of sdLDL-C, central obesity, and dietary pattern in Indonesia may be interesting.

ACKNOWLEDGMENT

The authors would like to thank to the National Institute of Health Research and Development (NIHRD) Ministry of Health, which offers the data. We also thank to IPB University for supporting this research.

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Jurnal Gizi Indonesia
(The Indonesian Journal of Nutrition)

Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition)
Vol. 11, No. 2, June 2023 (110-118)
Submitted: 27 November 2022, Accepted: 15 February 2023
Online https://ejournal.undip.ac.id/index.php/jgi

DETERMINING THE NUTRIENTS CHANGES AFTER UNDERGOING NUTRITIONAL COUNSELING AND COOKING ASSISTANCE AMONG T2DM OUTPATIENTS IN MALANG CITY, INDONESIA

Rany Adelina[1]*, Awalia Nanda Arianto[2], Sabrina Julietta Arisanty[1], Rafika Aprillia[3], Wisnowan Hendy Saputra[4], Risa Mafaza[1], Khairudin[1], Tapriadi[1], Rathi Paramastri[5]

ABSTRACT

Background: Nutrition and diets are critical factors for T2DM patients to maintain health. Nutrition education are considered less effective because most patients have not implemented them. This research tries to develop program innovation by combining nutrition counseling and cooking assistance for T2DM outpatients. This study aimed to know the risk factors for T2DM and *determine the effectiveness of programmed nutrition education (NEP) on changes in nutrient intake in patients with T2DM.*

Methods: A total of 70 participants registered as T2DM outpatients at Kedung Kandang primary healthcare center in Malang city. Subjects were recruited using a 'quota sampling' technique. The design of this study is a quasi-experiment study using a comparison of the control (n=32)-treatment group (n=38). This research was conducted from September to November 2018. Fifty minutes of intensive individual counseling and cooking assistance were provided to T2DM patients and families. The data were analyzed using independent sample t-test, Wilcoxon Mann Whitney U-test, and logistic regression. The patients' 4-d dietary records of 3 normal days and 1 holiday/weekend were assessed after 24 hours.

Results: From this study it can be seen that intake of amino acid lysine was significantly higher in the treatment group than the control group (p = 0.04). The intake of fiber, MUFA, and PUFA was greater in the intervention group, while sodium intake was lower in the intervention group. The risk factors of T2DM incidence were age (p = 0.036), education (p = 0.043), waist circumference (p = 0.015), and carbohydrate intake (p = 0.033).

Conclusion: T2DM patients treated with individual nutrition counseling and cooking assistance gained a higher intake of fiber, amino acid lysine, and unsaturated fatty acids. The most influential risk factors of T2DM incidence are age, education, waist circumference, and carbohydrate intake.

Keywords: *nutrition counseling, cooking assistance, nutritional intake, T2DM.*

BACKGROUND

Diabetes is the new plague of the 21st century developing throughout the world both in developing and developed countries with the most dramatic increase occuring in type 2 diabetes (T2DM), whose increase is mainly associated with an increase in the prevalence of sedentary lifestyle and obesity. T2DM is a metabolic disorder characterized by insulin resistance, dysfunction of β-cells, and increased risk of vascular diseases which can be caused and influenced by genetic, behavioral, and environmental factors[1,2]. More specifically, factors of age, lifestyle, and diet are causative to T2DM [3]. Certain types of food intake are associated with higher or lower insulin resistance and insulin secretion, which can increase the risks of diabetes and insulin resistance-related diseases [4].

Nutritional recommendations for T2DM patients set by the American Diabetes Association (ADA) include limiting energy intake to achieve or maintain ideal body weight; limiting total and saturated fat, cholesterol, and sodium; consuming adequate amounts of carbohydrates from nutrient-dense foods such as vegetables, fruits, whole grains, and legumes; and consuming enough fiber. Nutritional recommendations for

[1]Undergraduate Program in Applied Nutrition and Dietetics, Department of Nutrition, Politeknik Kesehatan Kementerian Kesehatan Malang, Indonesia
[2]Dietisien Profesional Education Study Program, Department of Nutrition, Politeknik Kesehatan Kementerian Kesehatan Malang, Indonesia
[3]D3 Nutrition Study Program, Department of Nutrition, Politeknik Kesehatan Kementerian Kesehatan Malang, Indonesia
[4]Department of Actuarial Science, Institut Teknologi Sepuluh Nopember, Indonesia
[5]School of Nutrition and Health Science, College of Nutrition, Taipei Medical University, Taiwan
[*]**Correspondence** : E-mail: rany_adelina@poltekkes-malang.ac.id

e-ISSN : 2338-3119, p-ISSN: 1858-4942

people with diabetes aim to prevent or slow down diabetes complications by achieving as close as possible to normal blood glucose levels and lipid profiles to reduce the risk of cardiovascular disease and blood pressure [5]. Although these nutritional recommendations are useful, the vast majority of people with diabetes do not achieve them and are at risk for dyslipidemia, hypertension, poor blood glucose control, and being overweight [5].

People with diabetes often find recommendations for healthy eating confusing and difficult to implement[6,7]. Barriers include a lack of understanding of dietary recommendations, lack of family support, limited budget, food choices, and cooking habits [8]. There is something dangerous about consuming excessive sugar. Excessive sugar intake is a major factor in the development of T2DM. People living with T2DM need a combination of medical care and a healthy lifestyle to maintain their condition and prevent complications.

The global prevalence of diabetes in 2015 was 415 million and is likely to grow to 642 million by 2040, with the most dramatic increase of 90 percent in T2DM following socio-cultural change [9]. The prevalence of T2DM in the aged fifteen population in Indonesia based on doctors' diagnosis data according to Indonesia Basic Health Research is 2% presently, with the highest average of 2.6% occurring in East Java [10].

Considering the mentioned nutritional problems, this study attempted to innovate nutrition programs through intensive nutrition counseling and cooking assistance for T2DM outpatients. The program was expected to give a positive impact on T2DM patients, especially in changes in nutrient-related intake. This study aimed to determine the change in nutritional intake of fiber, lysine, and unsaturated fatty acids after undergoing nutrition counseling and cooking assistance among T2DM outpatients in Malang city, Indonesia.

MATERIALS AND METHODS

Study Design and Participants

The study applied a quasi-experimental design with a control and treatment group. The control group was only given leaflets about the DM diet by a trained nutritionist, while the treatment group was given the same as the control group plus 1×50 minutes of intensive nutrition counseling (including booklet and slide presentations) and cooking assistance for one set of DM menu at lunchtime by expert nutritionist. The research was conducted from September to November 2018 at the primary healthcare center Puskesmas Kedungkandang of Malang City, Indonesia.

As many as 72 T2DM high-risk adults registered at the healthcare center voluntarily participated in the research, but 2 dropped out prematurely. Subjects were recruited voluntarily using a 'quota sampling' technique, out of the 70 participants, 32 were put in the control group while 38 were in the treatment. The participants were included in the study under certain criteria: literate, diagnosed with T2DM by the doctor at the Kedungkandang Healthcare Center, having classic symptoms of DM (polyuria, polydipsia, polyphagia, unexplained weight loss), Fasting Blood Glucose level (GDP) more than 126 mg /dL, plasma glucose level 2-hours Postprandial after Oral Glucose Tolerance Test (OGTT) more than 200 mg/dL, having a history of using Anti-Diabetic Drugs, and having complications of hypertension. Participants would be excluded under certain criteria: incomplete 4d-food records, worsening prognosis, using insulin therapy, and having infectious disease complications. Participant recruitment was voluntary. All subjects signed informed consent before participating in the study. The research protocol had been approved by the ethics test at the Health Research Ethics Commission of Politeknik Kesehatan Kementerian Kesehatan Malang with Reg. No.468/KEPK-POLKESMA/2018.

The study used research instruments of explanatory texts, informed consent, social demographic questionnaires to probe the participants' age, ethnicity, sex, educational level, occupation, and income per year. For anthropometric measuring instruments, stamped scales and Medline tape were used to measure body weight, height, and waist circumference. During the study, the control group was only given leaflets about the DM Diet, while the treatment group was given the same as the control group plus 1x50 minutes of intensive nutritional counseling and cooking assistance for 1 DM menu at one meal. The study used instruments of booklet and presentation slides containing counseling materials and recipes, a 4 days times 24 hours-food record form to evaluate nutrients intake and cooking ingredients. The data from the control group were collected by trained nutritionist enumerators (graduated from three years diploma program), while expert nutritionists (graduated from four years undergraduate program) handled the intervention group.

Data Analysis

The nutrient intakes data of each group were processed with the Nutrisurvey application and to be compared with the recommendations from the ADA. Data processing was carried out using descriptive statistical analysis and an independent sample t-test (if the data were not normally distributed, the Wilcoxon Mann Whitney U-test would be used) to compare the differences between the control and intervention. Logistic regression was performed to obtain Odds ratios (OR) to determine the association between nutritional intake and risk factor variables with T2DM and their interactions. The statistical test used the latest version of SPSS software at a 95% confidence level.

RESULTS

Socio-Demographic and Anthropometric Characteristics

The socio-demographic and anthropometric analysis results in Table 1 showed that the characteristics of the participants were female in the majority, married, Javanese ethnic, elementary school graduated, unemployed, under minimum wage income, abnormal waist circumference, and obese.

Tabel 1. Characteristic of Social Demographic and Anthropometric

Characteristics	Total (n=72)	Control (n=32) n (%)	Intervention (n=38) n (%)
Sex			
Female	55 (76.4)	24 (75)	29 (76.3)
Male	17 (23.6)	8 (25)	9 (23.7)
Marital Status			
Married	55 (76.4)	29 (90.6)	26 (68.4)
Widower	17 (23.6)	3 (9.4)	12 (31.6)
Tribe			
Java	60 (83.3)	30 (93.7)	30 (78.9)
Madura	11 (15.3)	2 (6.3)	7 (18.4)
Pendalungan	1 (1.4)	0	1 (2.7)
Educational Level			
Not in school	5 (6.9)	1 (3.1)	3 (7.9)
Elementary school	44 (61.1)	16 (50)	27 (71.1)
Junior high school	11 (15.3)	8 (25)	3 (7.9)
Senior high school	11 (15.3)	7 (21.9)	4 (10.5)
Diploma/Bachelor	1 (1.4)	0	1 (2.6)
Occupation			
Not working	32 (44.4)	14 (43.75)	16 (42.1)
Labor	4 (5.6)	3 (9.4)	1 (2.6)
Trader	16 (22.2)	5 (15.6)	11 (28.9)
Self-employed	5 (6.9)	1 (3.1)	4 (10.5)
Teacher	1 (1.4)	1 (3.1)	0
Tailor	4 (5.6)	3 (9.4)	1 (2.6)
Retiree	2 (2.8)	2 (6.3)	0
Masseur	1 (1.4)	0	1 (2.6)
Driver	1 (1.4)	1 (3.1)	0
Singers	1 (1.4)	0	1 (2.6)
Others	5 (6.9)	2 (6.25)	3 (7.9)
Monthly income			
Below the minimum wage	61 (84.7)	24 (75)	35 (92.1)
Equal to/Above the minimum wage	11 (15.3)	8 (25)	3 (7.9)
Waist circumference, cm			
Normal (P≤80, L≤90)	15 (20.8)	8 (25)	6 (15.8)
Abnormal (P>80, L>90)	57 (79.2)	24 (75)	32 (84.2)
Body mass index, kg/m^2	25.3±4.2	25±4.6	25.6 ± 4
Normal (18.5-22.9)	21 (29.2)	12 (37.5)	9 (23.7)
Overweight (23-24.9)	20 (27.8)	7 (21.9)	11 (28.9)
Obese (≥25)	31 (43.1)	13 (40.6)	18 (47.4)

Energy and Nutrient Intake

As seen in Table 2, there were significant differences in amino acids (lysine and glutamine) intake. Lysine intake in the intervention group was higher than in the control group. However, glutamine intake was lower in the intervention group. In addition, the intake of energy, fat, SAFA (Saturated Fatty Acids), protein,

and carbohydrates tended to be higher in the intervention group, although not significantly different. The intervention group had a higher intake of fiber, MUFA, and PUFA but a lower sodium intake.

Tabel 2. Energy and nutrient intakes in T2DM patients (n=70)

Intake	Control (n=32) Mean (SD)	Intervention (n=38) Mean (SD)	p-value
Energy (kkal)	1054 (322)	1158.9 (403.7)	0.24
Carbohydrate (g)	157.2 (58)	175.2 (75.2)	0.25
Fiber (g)	7.9 (3.3)	8.7 (3.5)	0.31
Fat (g)	32.6 (12.9)	35.1 (13.7)	0.36
SAFA (g)	15.1 (7.9)	15.8 (7.2)	0.68
MUFA (g)	7 (3.2)	7.1 (4.3)	0.89
PUFA (g)	7.3 (3)	7.9 (4.6)	0.93
Protein (g)	35.3 (11.8)	40.6 (14)	0.09*
AA glutamine (g)	0.15 (0.78)	0.04 (0.05)	<0.01*
AA lysine (g)	1.9 (0.6)	2.3 (0.8)	0.04†
Sodium (mg)	344.9 (320)	306.9 (306.7)	0.51

*Independent t-test and with significant p-value set at < 0.05.
†Wilcoxon Mann Whitney U-test with significant p-value set at < 0.05.

The Association Between T2DM and Risk Factors

Table 3. The association between T2DM and other factors and interaction

Predictor Variables	B	Sig.	OR
Age	2.434	.036*	11.407
Education	.765	.043*	2.149
Occupation	-.624	.092	.536
Waist Circumference by Sex	-.910	.015*	.403
Carbohydate Intake	.016	.033*	1.016
Constant	-.769	.696	.464

Based on table 3, on the age variable, it can be interpreted that persons aged 60 and above have 11.407 times greater chance of being diagnosed with diabetes and T2DM than those who are younger. On the education variable (the multinomial categorical independent variable), based on the OR value, it can be interpreted that people with a lower level of education have 2.149 times greater chance of being diagnosed with T2DM than those whose education was one level above. On the carbohydrate intake variable (continuous variable), it can be interpreted that for every 1 unit increase in intake given or occurs to a person gives the person 1.106 times greater risk of being diagnosed with T2DM. On the waist circumference variable, a female person with an abnormal waist circumference (>80 cm) has a 0.403 times greater chance of being diagnosed with T2DM.

DISCUSSION

The results showed that the majority of T2DM patients were obese with an average BMI > 25 kg/m^2. This is supported by previous research which states that the incidence of T2DM is increasing in general throughout the world, mainly due to an increase in the prevalence of a sedentary lifestyle and obesity. T2DM is influenced by genetic, behavioral, and environmental factors [1]. T2DM is increasingly common, mainly due to the increasing prevalence of sedentary lifestyles and obesity. Whether T2DM can be prevented by interventions that affect the lifestyle of subjects at high risk for the disease is unknown [11].

The patient's overeating behavior should be evaluated through a diet journal to establish a weight measurement that favors eating behavior. Behavioral improvement through motivational measures routine is considered effective in maintaining the desired behavioral change [12]. All patients should receive counseling and be encouraged to show greater adherence to nutritional therapy and treatment of T2DM [13]. Ramlan and Margawati (2016) said that providing intensive counseling can affect positive changes in a person's behavior [14].

Dietary intake, diet, and food quality have an important role in T2DM patients, by understanding the different characteristics of an individual can be used to develop and facilitate clinical practice to assist

diabetic patients in controlling their glucose [15]. Behavioral therapy that can reduce body weight and achieve effective glycemic control in T2DM patients is a modification to achieve and maintain long-term weight loss in obese T2DM patients [16].

Dietary recommendations for T2DM patients set by the ADA include limiting energy intake to achieve or maintain ideal body weight; limiting total and saturated fat, cholesterol, and sodium; consuming adequate amounts of carbohydrates from nutrient-dense foods such as vegetables, fruits, whole grains, and legumes; and consume enough fiber. In addition, a study conducted by Yuliani et al., (2020) showed that choosing functional foods such as yogurt containing probiotics is currently also an alternative for preventing secondary complications of T2DM that started much research [17]. Nutritional recommendations for people with diabetes aim to prevent or slow down diabetes complications by achieving as close as possible to normal blood glucose levels and lipid profiles to reduce the risk of cardiovascular disease; and blood pressure [5].

Although these nutritional recommendations are useful, the vast majority of people with diabetes do not achieve them and are at risk for dyslipidemia, hypertension, poor blood sugar control, and being overweight [5]. People with diabetes often find recommendations for healthy eating confusing and difficult to implement [6,7]. Barriers include a lack of understanding of dietary recommendations, lack of family support, limited budget, food selection, and cooking habits [8]. There is something dangerous about consuming too much sugar. Intake of too much sugar is a major key factor in the development of T2DM. People living with T2DM need a combination of medical care and a healthy lifestyle to maintain their condition and prevent complications [18].

Despite the recognition that diabetes and nutrition education is at the core of diabetes management, and that lifestyle education substantially proves a risk factor and delays the onset of diabetes in high-risk people, 6-8 Aboriginal people access diabetes and undernutrition education more than Australians [19]. A small study aimed at identifying effective nutritional interventions in Aboriginal communities [20]. The cooking program for diabetics aims to promote healthy eating by improving nutritional knowledge and cooking skills. A focus on healthy cooking techniques and the use of familiar and affordable foods is likely to increase its effectiveness [21].

Analysis of dietary records in patients with T2DM showed higher carbohydrate consumption in the intervention group than in the control group, this happened because respondents in the intervention group liked to consume simple carbohydrates such as sugar, syrup and sweet cakes. Types of simple carbohydrates (sugar, syrup, and sweet cakes) if consumed too much tend to cause overweight and obesity. In addition, carbohydrate food sources tend to be affordable for low-income groups, resulting in excess carbohydrate intake in the intervention group. Seligman et al., (2010) on their research said that to preserve caloric intake, food-insecure adults frequently shift their diets towards energy-dense, nutritionally-terrible foods (which include subtle carbohydrates, delivered sugars, and delivered fats), which diabetic sufferers are cautioned to keep away from to optimize their glycemic control. Such foods are less expensive than equicaloric portions of fruits, vegetables, and dairy products [22].

The advantage of this study was that it showed higher fiber (vegetable and fruit), amino acid lysine intake (wheat, eggs, fish, cheese and milk), MUFA and PUFA (omega 3 (mackerel and sardines) and omega 6 (cooking oil and nuts such as soybeans, almonds and cashews) sources of food) results in the intervention group than in the control. According to previous studies, the increase in lysine can increase the activity of GLUT4, which plays a role in the transport of glucose into glycogen. Sources of foodstuffs containing the amino acid lysine in wheat products, eggs, fish, cheese, and milk [23].

The increase in fiber also indicated that the participants consumed more vegetables and fruit. Meanwhile, the increase in MUFA and PUFA showed that the participants consumed more omega-3 and 6 sources of food. In addition, the sodium intake was lower in the intervention group. Sodium in patients with T2DM needs to be controlled regarding the development of the disease towards hypertension. The study showed that excessive sodium intake is independently associated with the risk of hypertension among a representative sample of Inner Mongolia residents [24].

Obesity is one of the main risk factors for T2DM [25], although increasing protein intake also has the potential to prevent T2DM. The optimal amount and quality of protein to prevent T2DM are controversial [26]. Although short-term interventions comparing low-protein diets have shown beneficial effects on weight loss, body composition, and several metabolic markers, the outcomes of long-term interventions are generally modest [27]. Furthermore, several prospective studies have raised concerns that even moderately high protein intake may increase the risk of T2DM [28], although associations have also been reported [29]. Several Similä et al. (2012) epidemiological studies have also suggested that replacing

protein with carbohydrates can reduce the risk of T2DM [30]. Contrary to short-term interventions, prospective studies indicate that high protein intake with a higher risk of T2DM is partly mediated through the effect of higher protein intake on obesity [28]. Relatively high protein intake was not independently associated with T2DM risk, but protein and carbohydrate quality modified risk when protein was consumed as a carbohydrate substitute. Supporting protein from plant sources and eggs from other animal sources may be beneficial in the prevention of T2DM [31].

The result of lower glutamine amino acid intake in the intervention group was also not expected. Sources of food containing the amino acid glutamine are animal dishes. This showed that the intake of these foods in the intervention group tended to be lower. The monthly income in the majority intervention group was below the minimum wage and the status of the unemployed was higher. It was possible because the intervention group could not afford to buy food ingredients as sources of the amino acid glutamine. Previous studies have shown that GLN supplementation can increase EPC mobilization and promote vascular endothelial repair in diabetic rats with limb ischemia [2].

Four-day food records were used to estimate food intake. In the week before each testing session, participants recorded all food and beverages, including portion sizes, consumed over three weekdays and one weekend day [32]. The Nutrition Data System for Research diet analysis software (Nutrition Coordinating Center, Minneapolis, MN, USA) was used to analyze food records to estimate mean daily intake of total energy (kcal/day), carbohydrates (g/day;%), protein (g/day;%), fat (g/day;%), fiber (g/day) and food groups [33]. 4-d food logs provide detailed information on diet and are not prone to memory errors, it may not be the best method for capturing occasionally consumed foods. The long follow-up time may have weakened the association between dietary protein and T2DM. However, the associations did not differ significantly in analysis with shorter follow-up times [31].

This study showed that there were some variables related to the risk of T2DM such as age, education level, carbohydrate intake, and waist circumference (female). The results of this study are in line with research conducted by Nayak et al. (2014) which states age to be the most influential risk factor of T2DM in Trinidad [34]. Increasing age can increase the risk of being diagnosed with DMT2. Previous research found the same result that T2DM in patients aged >45 years had a risk of 18.143 times compared to patients aged >45 years [35].

This study also found that a low level of education had correlation with incidence of T2DM. In a previous study, A person with a low educational level has risk of 2.53 times greater chance of being diagnosed with T2DM compared with a person with a high educational level [36]. Varying levels of SEP might contribute to the different vulnerabilities of diabetes through different pathways, including education, which affect the clustering of diabetes through health literacy [37]. The knowledge and skills attained through education may help people to reach a higher level of health consciousness, which, in turn, influence a person's choice of healthy food and healthy behaviors [38].

In this study, for every one-unit increase in intake given or occurs to a person, that person would be at 1.106 times greater risk of being diagnosed with diabetes mellitus and hypertension. The previous finding showed the same result, that higher dietary Glycemic Load (GL), Glycemic Index (GI), and carbohydrate, and lower dietary fiber increased the risk of T2DM in 37.846 Dutch adults aged 20–70 [39]. As recommended by the ADA in 1994, the priority in the planning of food intake and/or meals should be given to the total amount of carbohydrates consumed rather than the source of the carbohydrate. The previous study assumed that carbohydrate intake is the primary determinant of the postmeal glycemic response, and the response is similar if the carbohydrate is eaten separately or as part of a mixed meal [40]. High-GI diets can rapidly increase postprandial glucose levels, thereby increasing insulin demand. This may lead to pancreatic exhaustion. In addition, high-GI diets can increase postprandial free fatty acid release, directly increasing insulin resistance [41].

The other finding of this study was that waist circumference was correlated with T2DM and hypertension, especially in females. In previous research, waist circumference can diagnose T2DM higher than other indicators in women. This can be seen from the visceral deposition of fat and distribution of regional adipose tissue, changes in body fat distribution during menopause, postmenopausal accumulation of abdominal fat becomes more pronounced. Therefore, the relationship between female waist circumference and risk factors for T2DM is stronger [42]. The same result was also found by Jeon [43], that those who had high-increasing waist circumference levels over time had a higher risk of T2DM by 5–7 times. Zhang et al. (2017) study on sex differences in abdominal fat distribution on insight found that women have much more

hepatocellular lipids than men [44]. It is well-known that visceral adipose tissue is related to increased cytokine production and insulin resistance (IR) [45].

The limitation of this study was the unexpected result for energy, carbohydrate, protein, fat, and SAFA intakes which were greater in the intervention group. The other limitation of this study is there was no monitoring of food intake by enumerators in the control group; it could be that the control group consumed foods high in glutamine during the research process. In addition, there was no assessment of food intake before and after treatment in the control or intervention group. Further research is needed regarding outpatient T2DM intake by adding research variables such as biochemical data on blood sugar and HbA1c and increasing the number of subjects.

CONCLUSION

Combined nutritional counseling and cooking assistance program brings about a change in knowledge, attitudes, and nutritional intake in T2DM patients indicated by the results of four days of dietary records for 3 normal days and 1 day off/weekend for 24 hours. Such treatment can increase significantly the intake of fiber, amino acid lysine, and non-fatty acids among T2DM outpatients. Counseling and cooking assistance can also increase saturation. In addition, such intervention can reduce the sodium intake of T2DM outpatients, which means counseling and cooking assistance affect the nutritional intake of T2DM outpatients. Counseling and cooking assistance may not be able to increase glutamine amino acid intake, and unexpectedly not able to decrease carbohydrate intake, which means that there is no effect of counseling and cooking assistance on the nutritional intake of DM outpatients. The influential factors of T2DM are age, education, waist size by gender, and carbohydrate intake. Whereas age is the biggest risk variable, elderly people are at risk of T2DM.

ACKNOWLEDGEMENTS

Thank you to the participants who joined this study. Moreover, thanks to the enumerators for collecting the data. Lastly, this research was funded by Politeknik Kesehatan Kemenkes Malang in the year 2018.

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Jurnal Gizi Indonesia
(The Indonesian Journal of Nutrition)

Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition)
Vol. 11, No. 2, June 2023 (119-127)
Submitted: 10 January 2023, Accepted: 06 February 2023
Online https://ejournal.undip.ac.id/index.php/jgi

COMPARISON BETWEEN METABOLIC PARAMETERS, FOOD INTAKE, AND GUT MICROBIOTA IN TYPE 2 DIABETES AND NON-DIABETIC INDONESIAN WOMEN

Ninik Rustanti[1,3], Agnes Murdiati[1], Mohammad Juffrie[2], Endang Sutriswati Rahayu[1,4*]

ABSTRACT

Background: *Globally, the increasing incidence of type 2 diabetes mellitus (T2D) has resulted in an upsurge in research into this metabolic condition. Women, particularly in Indonesia, have a greater risk of T2D than males. The diversity of the gut microbiota (GM) in T2D is regulated by the number of carbs, protein, fat, and fiber consumed.*

Objectives: *This study examined the comparison between metabolic parameters, food intake, and GM in T2D and non-diabetic Indonesian women.*

Materials and Methods: *The cohort study included people who did not have T2D and those who did. On day 28 of observations, anthropometric, metabolic parameters, food intake, physical activity, and feces were collected. Feces were collected for pH, SCFA, and GM (L. plantarum, Bifidobacterium, and Prevotella) analysis.*

Results: *There were significant differences between non-diabetic and diabetic women in age, Waist Hip Ratio (WHR), fasting blood sugar (FBS), and HbA1c. The two groups did not differ significantly in terms of their macronutrient intake (calories, carbs, protein, and fat), total water, and dietary fiber. Fecal pH and GM did not statistically differ between the control and T2D groups. Fasting blood sugar and HbA1c were positively associated with age, duration of T2D, WHR, and total water consumption, but slightly negatively associated with dietary fiber intake. Fasting blood sugar was also slightly negatively associated with Prevotella, meanwhile HbA1c with Bifidobacterium. Carbohydrate intake were positively correlated with acetic, propionic, and butyric acid levels.*

Conclusion: *Macronutrient intake, fecal pH, SCFA, and GM did not differ because GM in T2D increased bacause metformin consumption so that SCFA similar between two group.*

Keywords *: food, gut microbiota, short-chain fatty acid, diabetes, women*

BACKGROUND

Diabetes Mellitus Type 2 (T2D) is a metabolic condition defined by elevated blood glucose levels caused by a combination of inadequate insulin production and insulin resistance [1]. The International Diabetes Federation (IDF) estimates that around 463 million (9.7%) adult persons aged 20–79 years had diabetes in 2019, with that number expected to climb to 700 million (10.9 percent) by 2045. Indonesia is one of the top ten nations in the world with the greatest prevalence of diabetes, with 10.7 million adult diabetics in 2019 and a projected increase to 16.6 million by 2045 [2]. The prevalence of diabetes among women is higher than among men in Indonesia based on the national basic health research in 2018 [3]. Compared to men, women had a greater relationship between diabetes mellitus and acute myocardial infarction, and chronic ischemic heart disease [4]

T2D in Indonesia is determined by lifestyle, eating behavior, eating patterns such as smoking, obesity, unhealthy diet, lack of physical activity, consumption of alcoholic beverages, hypertension, dyslipidemia, and risk factors that cannot change, such as age and genetic factors [5–7]. Evidence suggests that dysbiosis of the gut microbiota (GM) plays a critical role in the development and progression of T2D. Gut microbiota can disrupt the host's glucose homeostasis [8]. A prior study revealed a link between *Bifidobacterium* and diabetes mellitus development. *Bifidobacterium* were less represented in the microbiota of the diabetic group than the non-diabetic group [9–11]. The diversity of GM is regulated by the number of carbs, protein, fat, and fiber consumed, as well as the type of diet consumed [12–14]. *Prevotella*, which is primarily seen in persons in developing countries or vegetarians, is very prevalent in the gut microbiome of most Indonesians, according

[1] Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Indonesia
[2] Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia
[3] Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, Indonesia
[4] Center for Food and Nutrition Studies, Universitas Gadjah Mada, Indonesia
*** Correspondence**: endangsrahayu@ugm.ac.id

e-ISSN : 2338-3119, p-ISSN: 1858-4942

to the previous study [15]. *L. plantarum* is also a dominating bacterium in the GM of the average Indonesian [16]. Numerous metabolic products of GM, including short-chain fatty acids (SCFA) are implicated in the control of glucose metabolism [17]. Therefore, GM and its correlation with SCFA, metabolic markers, food intake has been considered as a suitable target for studying the T2D mechanisms. The purpose of this study was to compare between metabolic parameters, food intake, and GM (*L. plantarum, Bifidobacterium*, and *Prevotella*) in type 2 diabetes and non-diabetic Indonesian women.

MATERIALS AND METHODS

Study Design

This study was a cohort study compared two groups of subject participants: the non-T2D group and the T2D group. The primary outcomes in this study are food intake, GM composition, and fecal SCFA, whereas the secondary outcomes are demographic data, anthropometric data, metabolic markers, and physical activity. Demographic data including age, and duration of T2D were obtained at the start of this study. Anthropometric data, stool samples, and blood samples were collected at the end of this study. Further analysis of stool samples was performed for GM and SCFA analyses, while blood samples were for metabolic markers analysis such as HbA1c, fasting blood sugar (FBS), and cholesterol total analysis. For 28 days, food intake and physical activity data were obtained using a semi-quantitative food frequency questionnaire (SQ-FFQ) and the International Physical Activity Questionnaire (IPAQ). Based on the Indonesian Food Composition Data and Indonesians' eating patterns, the FFQ included 165 food items in 9 food categories. The individuals disclosed their consumption habits (never, daily, weekly, or monthly), as well as the quantity of every food item they had consumed. For further analysis, the stated daily consumption of each food item was translated to grams. The FFQ validated before to used. Consumption of macronutrients and micronutrients were determined using the NutriSurvey 2007 application. (http://www.nutrisurvey.de/).

Location and Time

The research was conducted in public health centers in the Sleman regency, Yogyakarta, Indonesia, and conducted from October 2019 to March 2020. FBS, HbA1c, and total cholesterol analysis were conducted in Parahita Laboratory, Yogyakarta. Preparation of DNA extraction and SCFA analysis was done in Biotechnology Laboratory and Waste Management Laboratory in the Faculty of Agricultural Technology, Universitas Gadjah Mada. The PCR analysis of gut microbiota was carried out at the Laboratorium Penelitian dan Pengujian Terpadu (LPPT), Universitas Gadjah Mada.

Subject Participants

The study compared two groups of subject participants: 12 non-diabetic women (the non-T2D group) and 12 women with T2D (the T2D group). Subjects with T2D were obtained from 133 women with T2D and 14 non-T2D women who visited 3 public health centers in Sleman regency, Yogyakarta, Indonesia. Flow diagram of study participant can be seen in Figure 1.

They were screened based on the inclusion criteria in this study. Subjects were recruited based on puskesmas visitor data. Then the data is screened based on medical records. if they are eligible, the subject is visited at his home to be interviewed and his nutritional status and fasting blood sugar are measured. if they met the inclusion criteria, subjects were asked to sign an informed consent. The inclusion criteria for the T2D group were as follows: women aged between 20 and 50 years old, BMI < 30, HbA1c ≥ 6.5%, not pregnant and/or breastfeeding, not menopausal, not smoking, not drinking alcohol, not consuming antibiotic drugs, and other drugs. The inclusion criteria for the non-T2D group were the same as for the T2D group, with except of having an HbA1c level of 6.5%. The exclusion criteria were: going through probiotic and/or antibiotic therapy within 28 days before drawing fecal samples, being pregnant, or withdrawal of consent during the study. At the end of the field study, only 22 women finished the study: 11 non-T2D women and 11 women with T2D. The subject who dropped out of the non-T2D group was excluded due to the consumption of antibiotics, while the subject from the T2D group dropped out because the subject did not consume anti-diabetic medicine.

e-ISSN : 2338-3119, p-ISSN: 1858-4942

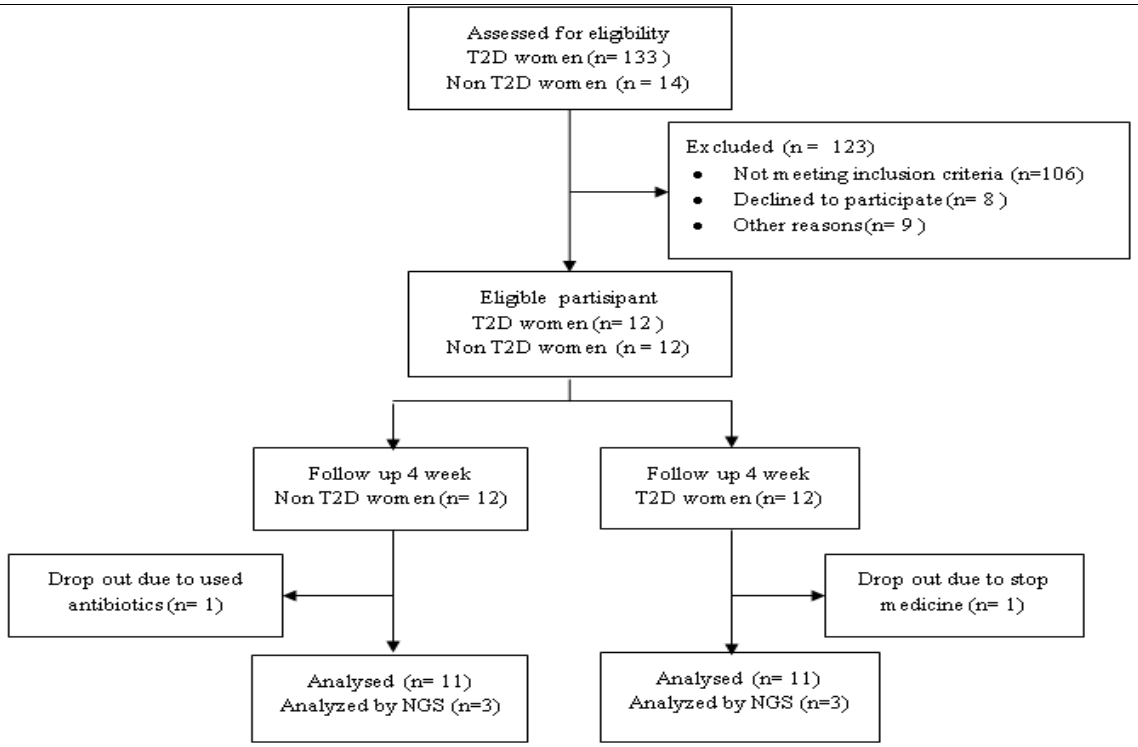

Figure 1. Flow diagram of study partisipant

Ethical Approval

As a condition of participation, all of the subjects had to provide written informed consent. The Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, authorized the study protocol, which followed the principles of the 1975 Declaration of Helsinki (Protocol number: KE/FK/1356/EC/2019; Approval date: 18 November 2019). The Ethical Committee approval document can be seen in Supplementary File 1.

Fecal Sample Collection

Before stool collection on day 28 (+1 day), each patient was given a fecal kit box and the method was described. The participants were asked to defecate and were then placed in fecal tubes. A sample in a fecal box containing ice bags was bought to the laboratory as soon as possible. After that, a fecal sample was immediately transferred into another fecal tube containing 2 mL of RNA (Sigma-Aldrich; R0901; Saint Louis, MO, USA). It was stored at frozen temperature (-18°C until -40°C) imamediately before it was used [15,18].

DNA Extraction

The sequencing process began with the extraction of DNA from the fecal sample. DNA was extracted using a modified bead-beating technique previously described by Nakayama et al [15] with adjustments as previously explained by Rustanti et al. [19].

Quantitative real-time qPCR Analysis

The microbiota analysis stage used the quantitative real-time PCR method, including DNA dilution from the results of DNA isolation, making PCR master mix, reading, making standard curves, and calculating the results of the total number of bacteria (log10 bacterial cells/g stool)[19,20]. The primers used had a DNA base sequence, as shown in Table 1.

Table 1. The specific primers used in the study

Target	Primer	Sequence (5' → 3')	
Lactobacillus plantarum	sg-Lpla-F	CTC TGG TAT TGA TTG GTG CTT GCA T	[20]
	sg-Lpla-R	GTT CGC CAC TCA CTC AAA TGT AAA	
Bifidobacterium	g-Bifid-F	CTC CTG GAA ACG GGT GG	[21]
	g-Bifid-R	GGT GTT CTT CCC GAT ATC TAC A	
Prevotella	g-Prevo-F	CACRGTAAACGATGGATGCC	[21]
	g-Prevo-R	GGTCGGGTTGCAGACC	

pH and SCFA Analysis

A pH meter was used to determine the pH of the feces (pH meter; Spear Eutech). Following calibration, the probe was dipped immediately into the feces sample and measured until a stable value was obtained [19].

Statistical Analysis

The SPSS 17 for windows was used for statistical analysis. Data presented as the mean ± SD. The data normality test was carried out using the Shapiro Wilk with a significance of 0.05 because it has a sample size of < 50. An independent t-test and non-parametric Mann-Whitney test were used to compare the groups with a significance of 0.05. Spearman correlation was used to examine a few parameters. Corrplot figure showing the correlation between two parameters analyzed using R.

RESULTS

Subject Characteristics

Subject characteristics after 4 weeks of observation are presented in Table 2.

Table 2. Characteristic of Subjects

Subject Characteristics	Non T2D (n=11) Mean ± SD	T2D (n=11) Mean ± SD	p-value
Age (years)	36.27 ± 5.69	43.82 ± 4.17	0.002[1]*
Weight (kg)	55.71 ± 5.76	5.68 ± 8.30	0.753[1]
Height (cm)	152.60 ± 4.77	150.62 ± 6.40	0.420[1]
Body Mass Index/ BMI (kg/m2)	23.94 ± 2.33	24.99 ±3.26	0.393[1]
Normal	7 (63.6 %)	6 (54.5 %)	
Overweight	4 (36.4%)	5 (45.5 %)	
Waist Circumference/WC (cm)	82.03 ± 5.70	85.96 ± 7.46	0.180[1]
Hip Circumference/HC (cm)	96.20 ± 4.67	95.03 ± 6,26	0.624[1]
WHR (Waist Hip Ratio)	0.85 ± 0.04	0.90 ± 0.05	0.011[1]*
Systolic	115.09 ± 10.80	124.45 ± 16.90	0.061[2]
Diastolic	77.73 ± 3.38	81.09 ± 9.69	0.489[2]
Fasting Blood Sugar /FBS (mg/dL)	81.09 ± 6.28	149.55 ± 53.58	0.000[2]*
HbA1c (%)	5.42 ± 0.39	8.19 ± 1.84	0.000[2]*
Total cholesterol (mg/dL)	175.64 ± 32.09	193.45 ± 39.81	0.261[1]
Duration of T2D (year)	-	2.5 ± 1.55	
< 1 year		2 (18.2%)	
1 - 3 years		6 (54.5%	
> 3 years		3 (27.3%)	
Type of drugs			
Metformin		2 (18.2%)	
Metformin & Glimepiride		8 (72.7%)	
Metformin & Gliabetes		1 (9.1%)	

[1] independent t-test; [2] Mann Whitney

Subject characteristics in T2D group had significantly higher age, FBS, HbA1c, and waist-to-hip ratio (WHR) than non-T2D group. Body mass index (BMI), cholesterol total, systolic, and diastolic were not significantly different between the two groups. The mean WHR in the T2D group (0.90 ± 0.05 cm) was higher than in the non-T2D group (0.85 ± 0.04 cm).

Food Intake and Physical Activity

The results of food intake and physical activity can be seen in Table 3. Food intakes per day, including calories, protein, fat, and dietary fiber, were not significantly different in the two groups. In both groups, only protein relative intake was in the range of WHO recommendations (protein 10% to 15%). Meanwhile, carbohydrate relative intake was lower (carbohydrate, 55% to 75%) and the fat relative ratio was higher than

the WHO recommendation (fat, 15 to 30%). However, the T2D group had a significantly higher total water daily intake (2073.36 ± 374.89 ml) than the non-T2D group (1401.13 ± 477.57 ml).

Table 3. Macronutrient Intake and Physical Activity Per Day in Two Groups

Macronutrient Intake and Physical activity	Non-T2D(n=11) Mean ± SD (% Energy)	T2D (n=11) Mean ± SD (% Energy)	p-value
Energy (kcal)	1395.66 ± 220.61	1453.55 ± 491.70	0.450[2]
Carbohydrate (g)	186.80 ± 45.94 (53.0)	186.66 ± 62.24 (51.6)	0.370[2]
Protein (g)	52.66 ± 13.87 (15.1)	46.75 ± 19.34 (12.9)	0.200[2]
Fat (g)	48.65 ± 12.15 (31.7)	61.46 ± 25.74 (37.5)	0.599[2]
Dietary Fiber (g)	13.22 ± 3.61	10.41 ± 6.87	0.061[2]
Total water (ml)	1401.13± 477.57	2073.36 ± 374.89	0.002[1]*
Physical activity (MET)	7354.6 ± 2618.8	6872.5 ± 3709.2	0.598[1]

[1] independent t-test; [2] Mann Whitney

Fecal pH and Short-Chain Fatty Acids (SCFA)

In the feces of the non-T2D and T2D groups, there was no significant variation in pH, total SCFA, acetic, propionic, and butyric acid (Table 4). Acetic acid was the most predominant SCFA. The acidity of the gut environment as a result of microbial metabolites was measured using fecal pH.

Table 4. The short chain fatty acid (SCFA) and fecal stool pH profile in two group

Characteristics	Mean ± SD		p-value
	Non T2D (n=11)	T2D (n=11)	
pH	6.17 ± 0.43	6.11 ± 0.45	0.778[1]
Total SCFA[a] (mmol/g)	21.04 ± 8.70	22.46 ± 10.42	0.732[1]
Acetic acid (mmol/g)	12.97 ± 5.36	12.89 ± 5.76	0.973[1]
Propionic acid (mmol/g)	3.87 ± 2.21	5.53 ± 4.26	0.562[2]
Butyric acid (mmol/g)	2.75 ± 1.21	2.75 ± 1.49	0.996[1]

[a]Total SCFA was the sum of acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, and iso-caproic acid.
[1] independent t-test; [2] Mann Whitney

Gut Microbiota

Table 5. Gut Microbiota between Two Treatment Groups

Type of microbial	Log10 bacterial cell/g (detection rate)		p value
	Non T2D (n=11)	T2D (n=11)	
L. plantarum	4.41 ± 0.29 (100)	4.72 ± 0.54 (100)	0.107[1]
Bifidobacterium	7.11 ± 0.47 (100)	6.72 ± 0.82 (100)	0.181[1]
Prevotella	7.85 ± 0.97 (100)	7.32 ± 1.35 (100)	0.302[1]

[1] independent t-test; [2] Mann Whitney

As the bacteria of interest, qPCR analysis was used to determine the counts of *L. plantarum, Bifidobacterium*, and *Prevotella* (Table 5). Compared to the T2D group, *L. plantarum* in the non-T2D group tended to be lower, but *Bifidobacterium* and *Prevotella* tended to be greater. There was no significant difference *in L. plantarum, Bifidobacterium,* and *Prevotella* in the T2D and non-T2D groups.

Correlations Between Metabolic Markers, Food Intake, SCFA, and Gut Microbiota

The correlation of the two investigated parameters using corrplot was seen in Figure 2. Both FBS and HbA1c showed a positive correlation with age (r: 0.498, p: 0.018 and r: 0.591, p: 0.004, respectively), duration of T2D (r: 0.838, p: 0.000 and r: 0.819, p: 0.000, respectively), total water (r: 0.456, p: 0.033 and r: 0.445, p: 0.038, respectively). Meanwhile they showed slightly negatively with dietary fiber intake (r: -0.397, p: 0.067 and r: 0.-363, p: 0.097, respectively). FBS had negative correlate with *Prevotella* (r: -0.400, p: 0.065), but HbA1c with *Bifidobacterium* (r: -0.417, p: 0.054). FBS positively correlate with HbA1c (r: 0.828, p: 0.033). HbA1c was positive correlation with WC and fat ratio (r: 0.442, p: 0.039 and r: 0.401, p: 0.065, respectively).

e-ISSN : 2338-3119, p-ISSN: 1858-4942

There was a positive correlation between total cholesterol and fat intake ratio (r: 0.502, p: 0.017), but a slightly negative correlation with carbohydrate ratio (r: -0.408, p: 0.060). Systolic displayed a positive correlation with total water (r: 0.467, p: 0.029) and a slightly positive correlation with duration of T2D, calorie and carbohydrates intake (r: 0.373, p: 0.082; r: 0.381, p: 0.080 and r: 0.387, p: 0.075, respectively).

Fecal pH displayed a negative correlation with WC (r: -0.460, p: 0.031), WHR (r: -0.430, p: 0.046), acetic acid (r: -0.501, p: 0.018), propionic acid (r: -0.574, p: 0.005), total SCFA (r: -0.493, p: 0.020). Acetic, propionic, and butyric acid acid was positively correlated with carbohydrate intake (r: 0.478, p: 0.024; r: 0.535, p: 0.010; r: 0.417, p: 0.053 respectively). Acetic acid had slightly negatively correlation with *Bifidobacterium* (r: -0.400, p: 0.065), meanwhile butyric acid had slightly positively correlation with *L. plantarum* (r: 0.363, p: 0.097) and calorie intake (r: 0.363, p: 0.097).

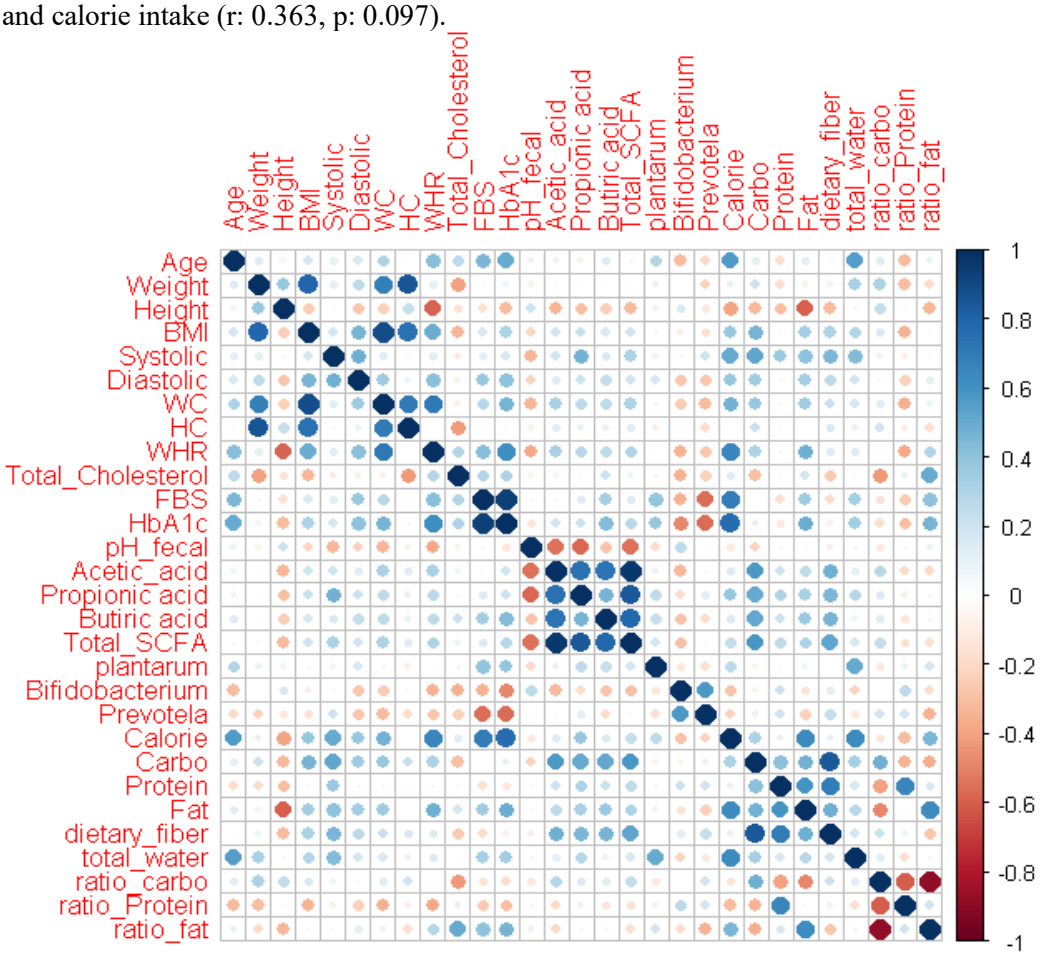

Figure 2. Corrplot Showing the Correlation between Two Parameters Analyzed using The Spearman Method. A Bigger Circle shows A Higher Correlation Coefficient. A Blue Circle Means Positive Correlation, While A Red Circle Indicates Negative Correlation.

DISCUSSION

There was no significant difference *in L. plantarum, Bifidobacterium,* and *Prevotella* in the T2D and non-T2D groups. Meanwhile, the relative abundance of Bifidobacterium, in the DM2 group was lower than the non-DM2 group. Bifidobacterium has a negative correlation with fasting blood glucose [22]. Bifidobacterium is often associated with protective properties in DM2 [10]. However, metformin significantly increased Bifidobacterium to improve glucose tolerance [23]. *Prevotella* levels tends to decreased in the T2D group. Because T2D was shown to have more *Bacteroides*, which has an antagonistic relationship with *Prevotella*, as seen in enterotypes, according to several studies [24]. Several studies have shown that there is a positive effect of metformin on the Bacteroidetes phylum, in particular increasing the abundance of Bacteroides, one of the genera within the Bacteroidetes phylum [25]. Another study shows that *Prevotella* level in lean T2D Indonesian subjects was lower than in the non-T2D subjects. In this study all T2D patients used metformin. some T2D patients in the overweight BMI category.

However, *Lactobacillus plantarum, Bifidobacterium,* and *Prevotella* in two groups were lower than *L.*

plantarum, Bifidobacterium, and *Prevotella* in the young healthy subject in Yogyakarta by about 5.0 ± 1.0 ; 9.4 ± 0.6 and 10.0 ± 1.2 log10 bacterial cell/g, respectively [16]. In the T2D group, *Lactobacillus plantarum* tended to be more prevalent than in the non-T2D group. *Lactobacillus plantarum* was shown to be the most prevalent among the higher-level *Lactobacillus* species detected in the gut.

Food intakes per day, including calories, protein, fat, and dietary fiber, were not significantly different in the two groups. Similar to another study, there were no significant differences in intake of total calories, carbohydrates, protein, or fat between the diabetes group and the control group [24]. The T2D group had a significantly higher total water daily intake (2073.36 ± 374.89 ml) than the non-T2D group (1401.13 ± 477.57 ml). Diabetic patients have symptoms including polyuria and polydipsia because of glucose homeostasis. For diabetics, drinking water can help to reduce your blood sugar (glucose) levels by diluting the amount of sugar in the bloodstream. Adequate intake of water also helps to alleviate the dehydration that comes with excess urination caused by high glucose levels[1,26]. In this study, water intake correlated positively with *L. plantarum*, but the mechanisms are still unclear.

In the feces of the non-T2D and T2D groups, there was no significant variation in pH, total SCFA, acetic, propionic, and butyric acid. These results are the same as a study in Japan which showed no significant difference in stool pH between the control group, namely 6.85 ± 0.85 and DM2, namely 6.74 ± 0.75 [27]. Stool pH under normal conditions ranges from 6.0 to 7.2 [28]. The pH of the stool indicates the acidity of the intestinal environment related to organic acids which are the result of commensal microbial fermentation in the colon such as acetic, propionic, and butyric acids [29,30]. It was proven in this study that the results of Spearman's correlation analysis showed a negative correlation between fecal pH and acetic acid (r: -0.501, p: 0.018), propionic acid (r: -0.574, p: 0.005) and total SCFA (r: -0.501, p: 0.018).

In DM2 patients who used metformin, SCFA increased again after decreasing in prediabetic and DM2 patients who did not use metformin [31,32].Propionic acid in the DM2 group, namely 5.53 ± 4.26 mmol/g, tended to be higher than non-DM2, namely 3.87 ± 2.21 mmol/g. This is because the relative abundance of Bacteroidetes which produce acetate and propionate in the DM2 group is significantly higher than the non-DM2 group. Firmicutes produces butyrate as a metabolic end product [8,29]. Acetic acid is the most dominant SCFA. The molar ratio of acetate, propionate and butyrate is approximately 60:20:20 [29]. Propionate has been demonstrated to cause the gut peptides glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) to secrete, which are implicated in hunger regulation, glucose metabolism, and inflammation [33]. The SCFA formed in the intestine is influenced by diet, the number and type of microbiota, and the transit time in the intestine. SCFA is mostly produced from anaerobic fermentation of dietary fiber and carbohydrates, especially resistant starch. r: 0.438, p: 0.042). Meanwhile, butyric acid has a positive correlation with dietary fiber (r: 0.456, p: 0.033)

A diet high in complex carbs and fiber can improve HbA1c and FBS because they can produce amount of producing bacteria short-chain fatty acids and produce mucus. The non-T2D group had a diverse gut microbiota that was enriched with short-chain fatty acid-producing bacteria and mucus-producing bacteria like *Faecalibacterium, Akkermansia, Lachnospira*, and *Roseburia*, which inhibited *Collinsella* and *Streptococcus* with proinflammatory effects and reduced inflammation in T2D. The presence of SCFA-producing bacteria can increased levels of intestinal SCFAs, reduced proliferation of dangerous bacteria, activated intestinal cells to release GLP-1, and increased insulin and HbA1c levels in patients [34].

This study has some limitations, which are listed below. The sample size was insufficient to provide significant statistical power, implying that further samples would be necessary to validate the study's findings.

CONCLUSIONS

The two groups did not differ significantly in terms of their macronutrient intake (calories, carbs, protein, and fat), total water, and dietary fiber. Fecal pH and GM did not statistically differ between the control and T2D groups. FBS and HbA1c were positively associated with age, duration of T2D, WHR, and total water consumption, but slightly negatively associated with dietary fiber intake. FBS was also slightly negatively associated with *Prevotella,* meanwhile HbA1c with *Bifidobacterium.* Carbohydrate intake were positively correlated with acetic, propionic, and butyric acid levels.

ACKNOWLEDGMENT

Thank for supported financially by the Ministry of Research and Technology (No. 3083/UN1.DITLIT/DIT-LIT/PT/2020), Grant for Dissertation of Doctorate Candidate (No. 27/E1/KPT/2020), and the Center of Excellence in Science and Technology (No. 6648/UN1/DITLIT/DIT-LIT/PT/2021).

e-ISSN : 2338-3119, p-ISSN: 1858-4942

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Jurnal Gizi Indonesia
(The Indonesian Journal of Nutrition)

Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition)
Vol. 11, No. 2, June 2023 (128-139)
Submitted: 23 March 2023, Accepted: 12 May 2023
Online https://ejournal.undip.ac.id/index.php/jgi

MALNUTRITION AT HOSPITAL ADMISSION AND ITS ASSOCIATED FACTORS IN INTERNAL MEDICINE INPATIENTS

Wita Rizki Amelia[1,2], Astrine Permata Leoni[1,2], Ahmad Syauqy[1*], Purwita Wijaya Laksmi[3], Martalena Br Purba[4], Etika Ratna Noer[1], Ari Wijayanti[2], Lora Sri Nofi[2], Befi Sundari[2], and Mita Arini[2]

ABSTRACT

Background: *Malnutrition at hospital admission may adversely affect patients' clinical outcomes. The Global Leadership Initiative on Malnutrition (GLIM) recently set a standard of measurable criteria to diagnose malnutrition.*

Objectives: *This study aimed to determine the proportion and risk factors of malnutrition at hospital admission.*

Materials and Methods: *A cross-sectional observational study was conducted in the internal medicine ward of the National General Central Hospital, Dr. Cipto Mangunkusmo (RSCM), Jakarta, from January to May 2022. Subjects aged 18 and above were recruited for this study. Malnutrition at hospital admission was defined according to the GLIM criteria. Then, the data were analyzed using multiple logistic regression to determine malnutrition risk factors, presented by odds ratios (OR) and 95% confidence intervals (CI).*

Results: *A total of 231 subjects were enrolled in the study. Among them, 85.3% were malnourished according to the GLIM criteria. In addition, subjects with a severe to total dependency on functional status (OR 9.406, 95%CI: 3.147–28.109), inadequate energy intake (OR 2.718, 95%CI: 1.197–6.172), and multimorbidity (OR 2.337, 95%CI: 1.045–5.228), were significantly associated with malnutrition at hospital admission cases.*

Conclusion: *According to the GLIM criteria, the proportion of malnutrition at hospital admission is high. The risk factors of malnutrition at hospital admission include low functional status, inadequate energy intake, and multimorbidity.*

Keywords *: Malnutrition; Nutritional status; Nutrition assessment; Hospital admission; Internal medicine, Inpatients*

BACKGROUND

The prevalence of malnutrition is around 15 to 60% globally[1]. Among patient admission to the hospital, the prevalence of malnutrition ranges from 31 to 73%,[2-6] with internal medicine inpatients mostly having the highest rate [5,7]. The number increased by 5% at discharge [2]. Several studies have proven that malnutrition may have a detrimental effect on clinical outcomes, namely in-hospital falls, longer hospital stay, higher admision cost, morbidity or complication or a critical care area neccessity, and mortality [4,8-14].

Malnutrition status at hospital admission was associated with physical function, hospitalization [15], polypharmacy [16,17], and comorbidities [5,18]. Other studies in hospital settings had also mentioned the associated factors of malnutrition or malnutrition at risk, involving age [17,19], gender [10], infections [20], cancer [6], multimorbidities [21], dementia, cognitive decline [19], depression [22,23], inadequate intake [24], gastrointestinal(GI) disorder [25], and medical procedure [26].

Various instruments to determine malnutrition status have emerged, such as the Subjective Global Assessment (SGA) score, which has become the gold standard [27]. However, the SGA malnutrition score is currently facing judgment issues regarding subjectivity which may affect its accuracy. The Academy of Nutrition and Dietetics–American Society for Parenteral and Enteral Nutrition (AND-ASPEN) and the European Society for Clinical Nutrition and Metabolism (ESPEN) have made a consensus that requires at least two clinical criteria to establish malnutrition status, in which AND-ASPEN criteria were proved to have higher accuracy [28]. However, the inter-population comparison is impossible due to the absence of global agreement regarding malnutrition criteria.

Responding to the challenge, Global Leadership Initiative on Malnutrition (GLIM) established a consensus that malnutrition diagnosis requires at least two criteria, including phenotypic (decrease in body weight [BW] or low body mass Index [BMI] or reduced muscle mass) and etiological (low food

[1] Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, Indonesia
[2]Nutrition and Food Service Unit, Dr. Cipto Mangunkusumo Hospital, Indonesia
[3]Geriatric Division, Internal Medicine Department, Faculty of Medicine, Universitas Indonesia, Indonesia
[4]Nutrition Unit, Dr. Sardjito Hospital, Indonesia
[*]**Correspondence** Email: syauqy@fk.undip.ac.id

e-ISSN : 2338-3119, p-ISSN: 1858-4942

intake/assimilation, or presence of inflammation) [29]. Unlike pre-existing methods, GLIM criteria are well-proven for substantial validity(30) and accuracy in predicting adverse clinical outcomes [30,31]. GLIM can detect 41.6% of malnutrition cases among inpatients in Brazil [30], supported by satisfactory accuracy as a result of a comparative study within several methods [28]. In Asia, a meta-analysis study has reported a significantly higher malnutrition rate based on GLIM criteria due to high diagnostic value [32].

Specifically in Indonesia, undernourishment was observed in 26.7–65.5% of adult internal medicine inpatients.(6,24,33) This number has recently tended to be higher when assessed using the GLIM criteria (75.0%) than the SGA (70.4%) [34]. However, the risk factors for malnutrition based on GLIM criteria have not been examined, particularly among inpatients in Indonesia, which has a large population with the highest mortality caused by comorbidity, namely stroke, ischaemic heart disease, diabetes mellitus, lung disease and liver disease [35].

It is critically important to evaluate risk factors for malnutrition at hospital admission in Indonesia due to the high malnutrition rate among internal medicine inpatients. Therefore, using the GLIM criteria, this study aimed to determine the prevalence and identify the risk factors among inpatients at admission to the internal medicine ward.

MATERIALS AND METHODS

An observational study with a cross-sectional design was conducted in the internal medicine ward of Dr. Cipto Mangunkusmo Hospital (RSCM), Jakarta, from January to May 2022. Dr. Cipto Mangunkusmo Hospital, a national general central hospital, is a well-known national referral center hospital with complete facilities and a high reputation as an educational hospital. This hospital supports the development of health professional human resources, including dietitians. The ethical review was obtained from the Faculty of Medicine Ethics Committee, University of Indonesia (1202/UN2.F1/ETIK/PPM.00.002/2021). In addition, each subject has signed an informed consent form before participating in this study.

The minimum sample size was calculated using the Roscoe formula (1982) for multivariate hypothesis test research; the minimum sample size was ten times the number of variables used in the study [36]. This study has fifteen variables, surpassing the minimum sample size to be analyzed. The minimum sample size in this study was 150 subjects. The inclusion criteria were patients with a minimum age of 18 years, patients who enter the internal medicine ward for a maximum of 48 hours, patients or caregiver who knows the patient's condition either before or during hospital admission, fluent in the Indonesian language, have the ability to understand instructions, and consent to be a subject. Patients were excluded if they were pregnant, could not be weighed (due to severe edema and/or unstable clinical condition), had incomplete data regarding nutritional status or medical history, and had incomplete limbs due to significant amputation or had significant skeletal growth abnormalities.

Three hundred three subjects were recruited using consecutive sampling at the beginning of treatment in the internal medicine ward from January until April 2022. Sixty eight subjects were excluded due to pregnancy (n = 1), could not be weighed (without any recall of weight loss and had normal muscle mass [n = 4], had moderate or severe edema/ascites and/or unstable clinical condition [n = 36]), had incomplete data regarding nutritional status or medical history (n = 30), had skeletal growth abnormalities due to history of juvenile idiopathic arthritis (n = 1). Finally, a total of 231 subjects were enrolled in this study.

A dietitian-nutritionist conducted the daily nutrition assessment for every new patient admitted to this hospital with at-risk malnutrition. The assessment includes malnutrition criteria based on the 2018 GLIM consensus, which requires at least one phenotypic and one etiologic criterion. Specifically, the phenotypic criteria in this study were collected by direct anthropometry measurement and interview, including non-volitional weight loss (> 5% within the last six months, or > 10% for > 6 months), low BMI (< 18.5 kg/m^2 in patients aged < 70 years, or < 20 kg/m^2 in patients aged $\geq$ 70 years),[9.14] and low muscle mass (ref. Asian Working Group of Sarcopenia [AWGS]) with cut-off points for low calf circumference (CC): male < 34 cm and female < 33 cm or low mid-upper arm muscle circumference (MAMC) (male < 21.1 cm, female < 19.2 cm) [38].

The etiologic criteria must consist of either reduced food intake (intake < 50% of energy requirement [ER] for > 1 week, or any reduction for > 2 weeks) or any presence of reduced food/nutrient assimilation (as identified if any occurrence of GI problem which might persistently adversely affect food intake or absorption, including dysphagia, nausea, vomiting, bloating, heartburn, gastrointestinal reflux disease [GERD], gastric cancer, diarrhea, constipation, pancreatic insufficiency, short bowel syndrome, hematochezia or any GI bleeding or any chronic intestinal insufficiency), or the occurrence of disease burden/inflammatory conditions

(if there is a diagnosis of the disease with chronic or acute inflammation, or supported by C-reactive protein [CRP] data > 5 mg/L as an inflammatory biomarker) [29,39,40].

The clinical parameters consist of medical history (previous surgery during the past five years, previous hospital admission within the past year, and history of the number of drugs consumed daily) [30], dietary history (energy intake) [41,42], and patient's medical status during hospital admission consist of comorbidity index [43], presence of cancer [30], presence of infection [20], GI problem [30], functional status (Barthel Index for Activities of Daily Living [B-ADL]) [44], and the presence of depression and/or dementi [19,22,23]. The primary data, including socioeconomic, dietary intake, and malnutrition status, were collected through direct interviews and measurements. The ward medical doctor assessed Charlson *Comorbidity Index*(CCI) to provide the comorbidity index at admission [43]. Functional status were assessed by ward nurse at admission, non-geriatric patients were collected directly, while geriatric patients were obtained from the medical record [44]. Other secondary data were collected by accessing the Health Information System (HIS) as an electronic version of the medical record, which contains complete data of patients treated at RSCM, including personal data (gender, date of birth and actual age), medical history, and current medical conditions (medical diagnosis, clinical conditions, and CRP).

Anthropometric data were obtained by direct measurements performed by trained personnel. In measuring body height and weight (BW), patients were asked to stand in a digital body stadiometer (SECA, China) while wearing very minimal clothes (precision: 0.1 cm for height and 0.1 kg for BW). Estimated BW was conducted if either edema or ascites were present by correcting the weight percentage based on the severity (mild 5%; moderate 10%; severe 15%), with an additional 5% reduction required when pedal edema bilaterally occurred [45]. BMI were determined by dividing the BW (kg) by height (m) squared. Weight loss was obtained by calculation (weight loss = [previous - actual BW]: previous BW x 100%), with the previous BW relying on subjects or caregiver's recall within the past six months or beyond. Other measurements included knee height (knee height caliper, Indonesia) with a precision of 0.1 cm, which was applied if the patient could not stand up. Then, we calculate the knee height with Shahar and Pooy's formula to obtain height prediction [46]. In addition, this study also included calf circumference (CC) with a precision of 0.1 cm (SECA, China), skin folds (SF) thickness measurement with an accuracy of 1 mm (Baseline, USA), and mid-upper arm circumference (MUAC) with an accuracy of 0.1 cm (SECA, China). Mid-upper arm muscle circumference (MAMC) data was obtained from the SF and MUAC data by calculation using the Nunes et al. formula [47].

At hospital admission, RSCM's trained dietitian-nutritionist assessed dietary history using the semi-quantitative-Food Frequency Questionnaire (FFQ) method to obtain each subject's food pattern, as validated by a previous study [48]. We then analyzed the nutrient intake using a web-based tool — Panganku (https://www.panganku.org, Indonesia) — to obtain daily dietary intake estimation before hospitalization.

All data were grouped into two categories based on references or median for statistical analysis. The factors including age were categorized as an older adult (≥ 60 years) or adult (18–59 years), gender as male or female, level of education as high school and below or higher education, income level as low or sufficient [49], history of surgery/invasive procedure as yes or no, previous hospitalized as yes or no, inadequate energy intake as yes (≤ 75% from daily ER) or no (intake of > 75% of daily ER) [42], drugs consumption before admission as ≥ 5 (polypharmacy) or < 5 (non-polypharmacy) kinds of drugs per day [16], comorbidity index as CCI score ≥ 5 (multimorbidity) or < 5 (not multimorbidity),(6) cancer as yes or no, presence of infectious diseases as yes or no, presence of gastrointestinal problem as yes or no, functional status as B-ADL score ≤ 8 (severe to total dependency) or > 8 which is according to median, and the presence of depression and/or dementia as yes or no.

The main outcome of this study was malnutrition status, defined by at least one phenotypic and one etiologic criterion. These criteria must meet the GLIM consensus, specifically for the Asia population requirement, as mentioned [29]. In the end, the nutritional status was categorized into two categories; malnutrition or normal.

Descriptive analysis was performed to determine the characteristic data. Bivariate analysis was performed using the chi-square test to assess the association between categorical independent variables and malnutrition status. Variables with $p < 0.25$ that have been considered clinically associated were applied to multivariate analysis using backward stepwise multiple logistic regression to identify malnutrition risk factors at hospital admission. The *p*-value < 0.05 indicated a statistically significant result. Odds ratios (ORs) and a 95% confidence interval (CI) were obtained. The IBM SPSS 23.0 statistical software was used for statistical analysis.

RESULTS

Malnutrition at hospital admission

Table 1 shows the subject's characteristics. Among all subjects, 85.3% were malnourished. The phenotypes of malnourished subjects were as follow: weight-loss (59.4%), low BMI (18%), and reduced muscle mass (90.9%). Etiological criteria in malnutrition group were established from reduced food intake/assimilation (77.2%), and the presence of disease burden/inflammatory conditions accounted for a larger percentage (98.0%).

Tabel 1. Characteristics of The Subjects According to Malnutrition Status

Parameter	Total	Malnutrition n=197	Normal n=34
Age (years), mean (SD)	54 (16)	55 (16)	45 (16)
Comorbidity Index (CCI), median (min-max)	4 (0–14)	5 (0–14)	2 (0–9)
Functional Status (B-ADL), median (min-max)	8 (0–20)	8 (0–20)	13 (4–20)
Nutritional Status, n (%)		197 (85.3)	34 (14.7)
Phenotypic Criteria, n (%)			
Weight Loss			
Yes		117 (59.4)	5 (14.7)
No		18 (9.1)	22 (64.7)
N/A		62 (31.5)	7 (20.6)
Low BMI			
Yes		35 (18)	0 (0)
No		53 (27)	34 (100)
N/A		109 (55)	0 (0)
Reduced muscle mass			
Yes		179 (90.9)	2(5.9)
No		18 (9.1)	32 (94.1)
Etiologic Criteria, n(%)			
Reduced food intake/assimilation			
Yes		152 (77.2)	18(52.9)
No		45 (22.8)	16 (47.1)
Disease burden/inflammatory condition			
Yes		193 (98.0)	25 (73.5)
No		4 (2.0)	9 (26.5)

B-ADL= Barthel Index for Activities of Daily Living; CCI=Charlson Comorbidity Index; N/A = Not Applicable; SD=Standard Deviation

Associated factors of malnutrition at hospital admission

Tabel 2 shows that the most affected factors was in the older adult group (93.3%) while other characteristics including male (87.6%), secondary school and lower educational level (86.2%), low-income level (84.8%), having surgery/invasive treatment before being admitted to the hospital (86%), having a history of being hospitalized (85.3%), having inadequate intake (89.7%), non-polypharmacy (86.9%), severe comorbidity index or multimorbidity (90.8%, the overall major chronic disease were tumour 26%, diabetes mellitus 14.3%, liver disease 10.8%), as well as the presence of certain clinical conditions including cancer (87.9%), infection (89.6%), having GI tract disorders (86.7% with dyspepsia 31.2% as the major symptom among all subjects), and severe to total dependency of functional status (96.6%), and depression and/or dementia (93.3%).

Tabel 2. Simple Logistic Regression of The Factors Associated with Malnutrition At Admission

Parameter	Malnutrition n (%)	Normal n (%)	OR(95%CI)	p-*value*
Demographic				
Age			3.469 (1.374–8.756)	0.010
Older adult (≥ 60 years)	84 (93.3)	6 (6.7)		
Adult (18–59 years)	113 (80.1)	28 (19.9)		
Gender			1.475 (0.709–3.070)	0.390
Male	106 (87.6)	15 (12.4)		
Female	91 (82.7)	19 (17.3)		
Socioeconomic				
Education Level			1.370 (0.594–3.160)	0.500
Secondary School or lower	156 (86.2)	25 (13.8)		
College	41 (82)	9 (18)		

Parameter	Malnutrition n (%)	Normal n (%)	OR(95%CI)	p-*value*
Income level			0.884 (0.389–2.011)	0.930
Low	140 (84.8)	25 (15.2)		
Sufficient	57 (86.4)	13 (13.6)		
Clinical paramater				
Previous surgery/invasive treatment			1.155 (0.554–2.406)	0.845
Yes	117 (86)	19 (14)		
No	79 (84.2)	15 (15.8)		
Previous hospitalized			1.004 (0.440–2.291)	1.000
Yes	145 (85.3)	25 (14.7)		
No	50 (85.2)	9 (14.8)		
Inadequate energy intake			4.071 (1.874–8.841)	0.0001
Yes (≤75% ER)	165 (89.7)	19 (10.3)		
No	31 (68.1)	15 (31.9)		
Drugs consumption before admission			0.601 (0.272–1.329)	0.294
≥ 5 (polypharmacy)	44 (80.0)	11 (20.0)		
< 5 (non-polypharmacy)	153 (86.9)	23 (13.1)		
Comorbidity index (CCI)			2.590 (1.197–5.602)	0.022
Severe (≥5)	109 (90.8)	11 (9.2)		
Not Severe (< 5)	88 (79.3)	23 (20.7)		
Cancer			1.356 (0.580–3.172)	0.618
Yes	58 (87.9)	8 (12.1)		
No	139 (84.2)	26 (15.8)		
Infection			3.280 (1.542–6.978)	0.003
Yes	155 (89.6)	18 (10.4)		
No	42 (72.4)	16 (27.6)		
Gastrointestinal problem			1.937 (0.795–4.719)	0.224
Yes	170 (86.7)	26 (13.3)		
No	27 (77.1)	8 (22.9)		
Functional status (B-ADL)			10.301 (3.495–30.362)	0.0001
≤8	114 (96.6)	4 (3.4)		
>8	83 (73.5)	30 (26.5)		
Depression and/or dementia			2.525(0.321–19.854)	0.704
Yes	14 (93.3)	1 (6.7)		
No	183 (84.7)	33 (15.3)		

B-ADL= Barthel Index for Activities of Daily Living; CCI=Charlson Comorbidity Index; CI=confidence interval; ER = Energy Requirements; OR=odds ratio.

Based on bivariate analysis, several factors were independently significantly associated with malnutrition at hospital admission, namely elderly (OR 3.469, 95%CI: 1.374–8.756, p = 0.010) with 70-years-old on average, followed by inadequate energy intake (OR 4.071, 95%CI: 1.874–8.841, p = 0.0001), multimorbidity (OR 2.590, 95%CI: 1.197–5.602, p = 0.022), presence of infection (OR 3.280, 95%CI: 1.542–6.978, p = 0.003), and severe–total dependency of functional status (OR 10.301, 95%CI: 3.495–30.362, p = 0.0001).

Multivariate logistic regression model of malnutrition at hospital admission

Potential risk factors for malnutrition are shown in Table 3. Based on the multivariate analysis, several factors were proved to increase the odds of malnutrition during hospital admission; the subject's functional status, inadequate energy intake, and comorbidity index.

Severe to total dependency on the subject's functional status (B-ADL ≤ 8) was the most significant risk factor for malnutrition cases (OR 9.406, 95%CI: 3.147–28.109), which was greater than the subject with mild to moderately dependent or independent functional status. Subjects with inadequate energy intake was significantly increasing the odds of malnutrition (OR 2.718, 95%CI: 1.197–6.172), meaning that subjects with insufficient energy intake pose a greater risk of having malnutrition at hospital admission than those with adequate intake. Lastly, patients with severe CCI during hospital admission might had greater odds of malnutrition in this study (OR 2.337, 95%CI: 1.045–5.228).

Thus, severe to total dependency on functional status (96.6%), inadequate energy intake (89.7%), and multimorbidity (90.8%) were the significant risk factors for malnutrition at hospital admission.

Tabel 3. Multivariate Logistic Regression Analysis of The Factors Associated with Malnutrition at Admission

Variable	B	OR	95% CI	p-Value
Functional Status (B-ADL ≤8)	2.241	9.406	3.147–28.109	0.0001
Inadequate Energy Intake (≤ 75% ER)	1.000	2.718	1.197–6.172	0.017
Comorbidity Index (≥ 5)	0.849	2.337	1.045–5.228	0.039

B-ADL= Barthel Index for Activities of Daily Living; CCI=Charlson Comorbidity Index; CI=confidence interval; ER = Energy Requirements; OR=odds ratio.

Description: involving independent variable of bivariate test results with a p-value < 0.25. Age, gastrointestinal tract disorder and infection were excluded during the backward stepwise multivariate regression analysis due to p >0.05.

DISCUSSION

This study found a very high malnutrition rate among inpatients of the internal medicine ward (85.3%), higher than most previous studies in Indonesia (26.7–65.5%) [6, 24,33]. However, their studies did not use GLIM criteria. Meta-analysis studies regarding hospital settings have showed that malnutrition prevalences range from 4% to 100% in Asia (>40% were reported by over 60% of studies), with SGA as the common tools being used.(50) Other studies reporting 44.2% were malnourished using the GLIM (6 of 20 studies held at hospitalized patients have reported malnutrition at a range of 30–90%) [32].

Apparently, previous studies have not distinguished between malnutrition at hospital admission or discharge. Moreover, the wide prevalence range might be due to different research methodologies and mixed populations. A greater malnutrition proportion at admission in present study has been confirmed by previous findings (GLIM 75% vs. SGA 70.4 %) [34], and supported by Syam et al. with malnutrition rate according to SGA have reached 65,5% among non-surgical inpatients [33]. This could be explained by the use of the GLIM which has 2 measurable criteria as the latest validated diagnosis tools which proved has better performance than previous methods in validity, and accuracy in predicting negative clinical outcomes [28, 30-32]. We also found that malnutrition have affected more on male group were similar to morbidity rate at national level [51], and low BMI (10.8%) were at most among male population in this country.(52) Furthermore, internal medicine inpatients were prevalent of malnutrition [5,7]. It might be due to the inflammatory conditions as proved by this study. These high-risk populations need early nutritional intervention for medical care management [42,53].

Subjects with severe to total dependency (B-ADL ≤ 8) on functional status have the highest potential risk factor for malnutrition at hospital admission, supported by a systematic review by Fávaro-Moreira et al. who sentenced that a general health decline including physical function were contributed [15]. It may be reasoned by an association between muscle mass and physical status among unhealthy adult [54], specifically malnourished patients were profiled as low of muscle mass, quality, strength and physical function [55].

Low functional status might be due to the reduction in muscle mass as it was mostly occure among malnourished group, and it was also affected by burden of disease as we found that multimorbidity was independently associated to malnutrition in this study, supported by Gn et al. (at risk of malnutrition had CCI median of 6) [56]. Moreover, muscle mass reduction have indicated a higher malnutrition rate among cancer patients when compared to using BMI, according to Sánchez-Torralvo et al [57].

Muscle mass were essential in determining sarcopenia which was defined by AWGS as “age-related loss of muscle mass, plus low muscle strength, and/or low physical performance” [38]. Even though this study using GLIM criteria which only consider muscle mass examination, decrease muscle strength could be another relevant parameters in nutritional assessment as investigated by Allard et al., low hand grip strength was related to malnutrition [18]. Other studies have found that poor hand grip strength worsens functional status [58].

A systematic review had concluded malnutrition factors including eating dependency among unhealthy individuals since they could have difficulty in daily activity including grabbing eating utensils and sitting autonomously [15]. Age factor might also explain this, the elderly group in present study were independently significantly associated to malnutrition, similar to previous research [59, 61]. This might be due to the fact that elderly with a significant low activity might experience reduction of muscle mass [25, 62, 63]. Furthermore, elderly patients with malnutrition at risk or malnutrition status were vulnerable to fall due to decreased muscle mass [14, 64]. Therefore, it has been recommended to evaluate muscle strength as a thorough assessment in sarcopenic risk patients [39].

This study also found inadequate intake had increased malnutrition rate by almost three times, as revealed by a previous study, it might be affected by appetite decrease [41]. This might occur since inadequate intake is recognized as an obvious direct factor of undernourishment which may result in nutritional status

decline, and is therefore taken into account when deciding malnutrition status. Inadequate intake could be a manifestation of diseases in which was influenced either by inflammatory conditions or any symptoms regarding alteration in GI tract function specifically in regards to nutrient digestion or absorption [29]. High prevalence of GI tract disorders among the malnourished group based on our findings might be the reason.

The inflammation occurrence either in acute on chronic diseases might negatively impact nutrition intake or utilization. This issue could possibly supported as we have found the contribution of multimorbidity in developing malnutrition status, this was inline with previous observation [5]. Even though only limited studies regarding association of low intake and high CCI, the elderly patients which was at risk of multimorbidit [65] was founded to have lower intake among malnutrition group [66]. We may confirmed this due to the association of age and malnutrition were revealed.

Even though infection as a presence of acute inflammatory were not contributed to malnutrition case in this study, it was correlated to malnutrition status on present. Though limited studies regarding infection as a malnutrition risk factor at hospital admission, Fitzpatrick et al. discovered that Healthcare-Associated Infection (HCAI) raises the malnutrition risk among inpatients [67]. Infection and malnutrition are well-known as vicious cycle associations. Infection may cause low nutrient intake or assimilation or increase the catabolic state due to inflammation [68], this might explain our findings. Either inadequate intake and catabolic phase can affect muscle mass reduction[29,39,69], or nutrient intake/assimilation could be affected by pharmacotherapy [16,17], as proven by Graeb et al. that the presence of infection alone were associated with a higher intake insufficiency [70].

Another possible factors of malnutrition might be educational levels as proven by previous research [71,72]. Unfortunately this is contrary to our findings as a slight variation among the malnourished group might be the reason. The current insignificant findings regarding the association of income levels and malnutrition might also related to this, contrary to the previous research [72]. We may hypothesize that education and income levels which were possibly co-linear in influencing the food preferences were not a significant factors among our respondents as health issues were at most affecting the respondents nutritional status as previously mentioned. Nevertheless, several other factors which found insignificant in present study were necessary to be evaluated in future investigation as mentioned by O'Keeffe et al., finding determinant factors of malnutrition among adult patient in particular [73].

To the best of our knowledge, this study is the first to investigate malnutrition, using GLIM criteria, and its associated factors in internal medicine inpatients in Indonesia. A large number of subjects enrolled in this study might represent malnutrition proportion in this population. In addition, nutritional assessment in this study were performed by trained personnel with equivalent understanding, and the population studied were specifically only newly admitted inpatients. Several factors that have been proven to influence the development of malnutrition at admission might be considered during initial assessment in daily practice. Despite the advantages of this study, we had limitations due to difficulty in obtaining weight data because not all patients capable to stand up as well as cut-off points were not established regarding muscle mass for certain conditions (severe edema) in particular. Therefore, we maximized by using validated anthropometric measures, bed scale and chair scale for BW, while calf circumference were used to determine muscle mass reduction as recommended. Assimilation of food was also maximized by using medical diagnosis and clinical data, as the cut-off point of frequency, intensity, duration, and severity of GI tract disorders were still unavailable.

CONCLUSIONS

This study found a higher malnutrition rate among inpatients at hospital admission. Low functional status is the most significant risk factor for malnutrition, followed by inadequate energy intake, and multimorbidity. A large number of malnutrition cases compared to previous studies reveals the importance of using valid malnutrition assessment tools, the needs of effective treatment of malnutrition, and further investigation regarding the clinical impact of malnutrition at hospital admission.

ACKNOWLEDGMENT

We would like to thank all parties who also support the implementation of this research, including the Education Fund Management Institute of the Republic of Indonesia (LPDP RI), RSCM, Geriatric Division and Hepatobiliary Division of the Department of Internal Medicine, Faculty of Medicine, University of Indonesia, Integrated Inpatient Services Building A. Nutrition Installation and Food Production along with the following Nutritionist-Dietitian staff : Fitri Hudayani; Martha Susanty; Dhi Ajeng KW; Maya R; Kurniasih AP; Yudhi A; Firlianita AA; Finabilla CA; Annisa AR; Lulu O; Asri P; Dedek PBS; Ebigail D; Aisyah PR; Vinia RN; Silvia IS; Audrey MSA; Irhamna S; Sintia IA; Nurul A; Immatul F; Vyanty H; Lilik FA; Desy I. All nurses

and medical doctors. All respectfull colleagues specifically Nur CB, Delita S, Utami S, Nove ZW, and Dipo WK.

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