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Assessing the Prevalence of Dyslipidemia in Apparently Healthy Urban Obese Adults Residing in South Delhi, India

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ABSTRACT

The present research study was conducted to assess the prevalence and pattern of dyslipidemia in apparently healthy urban obese adults residing in South Delhi. Dyslipidemia and obesity are two prominent contributing risk factors for cardiovascular disease. This cross-sectional study was conducted among 150 apparently healthy obese adults with a body mass index of ≥25 kg/m^2. Data regarding the socio-demographic characteristics as well as anthropometric parameters were collected. To evaluate serum lipid levels fasting blood samples were collected by trained technicians and analysis was carried out in a certified laboratory. Dyslipidemia was defined as the presence of hypercholesterolemia, high levels of Low-Density Lipoprotein (LDL) cholesterol, and /or lower concentration of High-Density Lipoprotein (HDL), present alone or in combination. The overall prevalence of dyslipidemia was noted to be 78%. The prevalence was observed to be much higher in the obese female subjects (81.43%) than the obese males (75%). Mean triglycerides levels were significantly higher in the obese men than females while, HDL–C levels were higher in the female subjects ($p<0.05$). Further, it was seen that low concentration of HDL was the most prevailing deranged lipid parameter (52.67%) followed by elevated triglycerides levels (49.33%) and elevated LDL levels (39.33%) in these obese adults. The study thus highlights the importance of regular and timely screening for apparently healthy populations. Advocating suitable and timely medical and dietary interventions can help in both monitoring and avoiding further health-related complications.

Keywords: cholesterol, dyslipidemia, high-density lipoprotein, obesity, the prevalence

INTRODUCTION

World Health Organization defines overweight and obesity as 'abnormal or excessive fat accumulation that may impair health' (WHO, 2019). Obesity is an independent risk factor for dyslipidemia, Type2 Diabetes, and Coronary artery disease and is rising in prevalence throughout the developed and developing countries (Jacob *et al.* 2014). In India obesity is emerging out to be a major health threat.

In India, the prevalence of obesity is increasing rapidly. In urban areas, the prevalence of obesity ranges from 13–50% while in rural areas the prevalence rate ranges from 8% to 38% (Mahajan & Batra 2018). According to the National Family Health Survey -NFHS-4 (2015-2016) data the prevalence of obesity in India among adults (15–49 years) is reported to be 20.6% in women and 18.9% in men (IIPS 2017) . In Delhi, the prevalence rate is much higher than the national prevalence (33.5% and 26.4% among women and men respectively). According to ICMR-INDIAB study, the prevalence of obesity and central obesity varied from 11.8% to 31.3% and 16.9%–36.3% respectively across three states and one union territory in India (that is, Jharkhand, Maharashtra, Tamil Nadu & Chandigarh respectively; Pradeepa *et al.* 2015).

The rising prevalence is associated directly with obesity-related co-morbidities like high blood pressure, metabolic syndrome, dyslipidemia, diabetes mellitus (type 2), and cardiovascular disease (Jacob *et al.* 2014; Dewi 2007). Aberrations related to cholesterol lipoproteins such as elevated cholesterol, VLDL, LDL and triglycerides and/or low HDL levels are major risk factors related to Coronary Heart Disease (CHD) (Gupta *et al.* 2017). Dyslipidemia, a group of biochemical disorders, is frequently encountered in obese individuals (NCEP 2002). Dyslipidemia is defined as the presence of any of the lipid

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aberrations existing alone or in the combination that is: elevated levels of TC or LDL-C or serum TG or a lower level of HDL-C" (Chandra *et al.* 2014).

Abnormalities in lipid metabolism are very common in individuals who are obese. Approximately 60–70 percent of patients with obesity have dyslipidemia (Feingold & Grunfeld 2000). Obesity-associated dyslipidemia undoubtedly plays a significant role in the development of atherosclerosis as well as CVD in obese individuals (Howard *et al.* 2003). Obesity, a modern world pandemic, is closely linked to dyslipidemia, which is mainly driven by the effects of insulin resistance and proinflammatory adipokines. Nevertheless, recent evidence indicates that obesity-induced dyslipidemia is not a single pathophysiological phenomenon, but has unique characteristics depending on many varying factors (Vekic *et al.* 2019). Elevated levels of triglycerides and low levels of HDL-C are metabolically interrelated. Amongst South Asians, this metabolic phenomenon is further associated with elevated levels of small LDL particles regardless of comparatively normal levels of LDL-C (Bilen *et al.* 2016). This is accompanied by a common metabolic derangement known as 'atherogenic dyslipidemia'(Patel *et al.* 2010; Sharobeem *et al.* 2007; Enas *et al.* 1996; Misra & Khurana 2010).

The aim of the present study was thus to assess the prevalence and pattern of dyslipidemia in apparently healthy obese adults in the urban population of South Delhi.

METHODS

Design, location, and time

The present cross-sectional study was conducted in South Delhi, India. Participants were recruited from South Delhi by holding health camps at different locations after taking necessary permissions from the concerned authorities which were primarily Resident Welfare Association (RWA) of the localities. These health camps were organized with the help of RWA's and local health practitioners of the concerned areas. Purposive sampling was done to recruit apparently healthy obese adults who volunteered to be part of the study based on the inclusion criteria. The data were collected from November 2016 to September 2017.

Sampling

The present cross-sectional data is a part of another ongoing study that is being conducted on 400 subjects. Data from a sub-sample in relation to dyslipidemia is being reported here for 150 apparently healthy, free-living; obese adults comprising of both males and females. The sample size for the present study was determined using formula:

$$n= z^2p(1-p)/d^2$$

$$Z^2= (Z\alpha+z\beta)^2=95\%$$

p: Prevalence (10%) (Prevalence of dyslipidemia in Indian adults = 10%, (Ranganathan *et al.* 2015; Misra *et al.* 2004).

The apparently healthy obese adults belonging to the age group of 35–50 years who were willing to participate in the study were recruited. Previous studies indicate that the cardiometabolic risk factors are high in middle-aged adults therefore this age range was selected. Further, obese individuals are often at a higher risk of developing cardiometabolic disorders and thus their dyslipidemia treatment is often indicated. So, it is important to assess the cardiometabolic risk among these subjects.

Subjects with a history of any chronic disease like hypertension, diabetes, etc. or those on medications for hypertension, diabetes or dyslipidemia; as well as pregnant and lactating women were excluded from the study.

Data collection

Information related to socio-demographic characteristics like gender, religion, age, family set up and marital status was collected using a pre-tested self-administered questionnaire.

Anthropometric measurements. Weight and height were taken following standard procedures. Weight was taken using a calibrated electronic bathroom scale. To assess the accurate weight of the participants, it was ensured that the shoes and any heavy clothing were removed. They were asked to remove other heavy objects on them or any such things in their pockets (like mobile phones, wallets, coins, heavy jewellery, watches, etc.). Weight was measured first thing in the morning before breakfast when subjects were in fasting state. For measuring height, commercial stadiometer was used. Height was measured using standard protocols in centimetres (to the nearest 0.1 cm). Obesity was defined using Asian criteria

to define obesity i.e. BMI of ≥25 kg/m^2 among both the genders (Misra *et al.* 2009).

BMI (kg/m^2) = weight (in kg)/height (in meters, square)

The non-stretchable fibre-glass tape was used to measure the waist and hip circumference to the nearest 0.1cm. ference to the nearest 0.1cm.

Hip circumference. Was measured at the maximum width of the buttocks and the waist measurement was taken at the point which is mid-way between the inferior margin of the last rib and the crest of the ileum in a horizontal plane passing the tape snugly but not tightly. All anthropometric measurements were taken twice and the mean of the two readings was used for analysis. Using anthropometric measures, anthropometric ratios like 'waist to hip' and 'waist to height' ratios were calculated using standard formulas:

Waist to hip ratio = waist circumference (cm)/ hip circumference (cm)
Waist to height ratio = waist circumference (cm)/height (cm)

For both male and female subjects, 0.5 was used as reference cutoff for the waist to height ratio (Ashwell & Gibson 2016) and WHO-Asian cutoffs that are, 0.85 and 0.90 were used as reference cutoffs for the waist to hip ratio for female and male subjects respectively (WHO 2011).

Biochemical assessments. Blood samples of the study participants were withdrawn after overnight fasting 8–10 hours by a trained technician and the analysis was carried out under standardized procedures in a well-established ISO 9001: 2001 certified laboratory. Lipid parameters were determined using the following methods: 1. Cholesterol-CHOD-PAP method: The concentration of serum total cholesterol was determined by modified Roeschalu *et al.* 1974. Principle Cholesterol esterase converts cholesterol esters to cholesterol and fatty acid. Cholesterol Oxidase (CHOD) and Peroxidase (POD) catalyses the conversion of cholesterol and hydrogen peroxide to red colour quinoneimine dye. The absorbance of quinoneimine measured at 505 nm is proportional to cholesterol concentration in the specimen; 2. Triglyceride-GPO Trinder method: The serum triglyceride was determined by the enzymatic method, employing lipoprotein lipase, glycerol kinase, Glycerol Phosphorase Oxide (GPO) and peroxidase (McGowan *et al.* 1983); 3. HDL-Phosphotungstic acid and Mg2 + ions directly precipitate low-density lipoprotein & VLDL and can then be extracted by centrifugation. High-Density Lipoprotein (HDL) remain supernatant. HDL cholesterol is estimated in the supernatant, using cholesterol working reagent, by a series of enzymatic reactions which are induced by the cholesterol oxidation into Cholestenone by cholesterol oxidase, followed by the formation of hydrogen peroxide. Further, 4-aminoantipyrine and phenol react with hydrogen peroxide to form red-coloured quinoneimine in a second reaction catalysed by peroxidase (Burstein & Allison 1970); 4. LDL- LDL cholesterol was estimated by the Friedewald calculation from fasting measurements i.e,

LDL = TC - (HDL + Triglycerides/5)
(Friedewald *et al.*1972)

Dyslipidemia. Dyslipidemia was defined as' a presence of any of the abnormalities, occurring alone or in a combination-increased concentration of TC or LDL-Cor serum TG or a decreased concentration of HDL-C' using NCEP (2002) guidelines: 1. Hypercholesterolemia – cholesterol levels (serum) of ≥200 mg/dl; 2. Hypertriglyceridemia–triglyceride levels (serum) of ≥150 mg/dl; 3. Low HDL cholesterol – HDL cholesterol levels of <50 mg/dl for femlaes and <40 mg/dl for males; 4. High LDL cholesterol – LDL cholesterol levels of≥130 mg/dl calculated using the Friedwald's equation.

Ethical approval. The study protocol was approved by the Institutional Ethics Committee, Institute of Home Economics, University of Delhi (Ethics No: IHE/2016/Ethics/018). Written informed consent was taken from all the participants in English as well as in Hindi.

Data analysis

Data were double entered in Microsoft Excel 2007. For statistical analysis of the data Statistical Package for Social Sciences (SPSS) version, 25 was used. A two-tailed 'p' value was used for calculating statistical significance; a value of $p<0.05$ was taken to be significant. Descriptive statistics were calculated for

quantifiable variables (means and standard deviation). Comparison of means between the male and female participants was done using the t-independent test. For assessing the significance of the categorical variables chi-square value was computed.

RESULTS AND DISCUSSION

The socio-demographic characteristics of the study participants revealed that out of the total study subjects enrolled (n=150), 53% (n=80) were males and 47% (n=70) of the subjects were females. The mean age of the study participants was 41.36±4.75 years (viz. 41.53±4.64 years for male subjects and 41.19±4.91 years for female subjects). Majority of the participants were vegetarians (52.66%) while the rest 47.33 % were non-vegetarians. Among vegetarians, the proportion of lacto-vegetarians was higher than the Ovo-Lacto vegetarians (72.15% and 31.64 % respectively). The nuclear family set up was predominantly seen as 66 % of the subjects belonged to nuclear families while only 34% had a joint family setup. The data is typical of an Indian urban setup. As far as religion is considered, the majority of the participants were Hindus (69%) followed by others like Muslims (13%), Christians (17%) and Sikhs (1%).

Table 1 highlights the anthropometric profile of the respondents. The mean BMI of the study subjects was reported to be 27.77±2.68 kg/m^2 (28.4±3.28 kg/m^2 vs 27.06±1.53 kg/m^2 for male and female subjects respectively). Since the subjects recruited were all obese, this is well depicted in their distorted anthropometric profile. Means for anthropometric indices like waist to height and waist to hip ratio were higher than the reference cut off points for both the genders.

The mean values for the lipid parameters that is, total cholesterol, triglycerides, and LDL for the total study subjects were reported to be 173.22±24.73 mg/dl, 164.52±64.79 mg/dl, 96.91±25.22 mg/dl respectively. Mean HDL for male and female subjects was 42.40±4.56 mg/dl and 44.58 mg/dl respectively. Gender wise stratification of the results (Table 2) showed that there existed a significant difference in the mean triglycerides (p-0.04*) and HDL levels (p-0.000*) among obese male and female subjects.

The modified National Cholesterol Education Program–Adult Treatment Panel-III (NCEP 2002) reference values were applied to classify lipid parameters and assess dyslipidemia. In the present study, the prevalence of dyslipidemia was assessed to be 78% (Figure 1).

The prevalence was higher in obese female subjects in comparison to the obese male subjects (that is, 81.43% and 75% respectively). The difference, however, was statistically insignificant (p-value 0.34). The results are of great concern as the subjects were apparently healthy with no prior history of any chronic diseases.

Figure 2 further distributes subjects according to the abnormal lipid parameter. The prevalence of elevated total cholesterol, triglycerides, low high-density lipoprotein cholesterol and low-density lipoprotein cholesterol was observed to be 12.66%, 49%, 52.66%, and 39.33% respectively. In females, low HDL was the most prevalent deranged lipid parameter (81.43%) followed by elevated triglycerides (41.43%) and high LDL levels (40%). In the case of men, however, high

Table 1. Anthropometric profile of the subjects

	Males (n=80)	Females (n=70)	p-value
Age (years)	41.53±4.64	41.19±4.91	0.66
Height (cm)	165.5±8.52	155.76±6.21	<0.001*
Weight (kg)	77.83±8.52	65.69±5.57	<0.001*
Waist circumference (cm)	94.04±11.77	91.24±6.41	0.07
Hip circumference (cm)	102.42±8.54	104.46±5.71	0.09
Body mass index(kg/m^2)	28.4±3.28	27.06±1.53	0.002*
Waist /hip ratio	0.92±0.07	0.88±0.07	0.000*
Waist /height ratio	0.57±0.07	0.59±0.04	0.08

Table 2. Lipid profile of the subjects

Lipid parameter (mg/dl)	Males (n=80)	Females (n=70)	p-value
Cholesterol	173.96±25.42	172.38±24.10	0.69
Triglycerides	174.32±71.26	153.32±54.90	0.04*
High-density lipoprotein	42.40±4.5	44.58±5.56	0.00*
Low-density lipoprotein	96.87±24.61	96.96±26.09	0.98

triglycerides levels (56.25%) followed by high LDL (38.75%) and low HDL levels (27.50%) were the most common deranged parameters. It was interesting to note here that high cholesterol was not common among these obese adults. Only 12.67% of the total subjects were reported to be hypercholesterolemic (14.29% and 11.25% of males and females respectively). Figure 3 further distributes subjects with dyslipidemia for the presence of several abnormal blood-lipid parameters as per NCEP ATP III guidelines. As many as 29.91% of the total obese adults had a presence of any one of the lipid parameter that was deranged (viz. 36.67% in men and 22.81% women).

Almost half of them (49.57%) had the presence of any two abnormal lipid parameters, while 5.98% of them had abnormalities in all the four lipid parameters. A similar pattern was observed when data were stratified based on gender where the majority of obese men (52.63%) and women (46.67%) had the presence of any two abnormal lipid parameters. Greater number of female participants had the presence of any 3 abnormal lipid parameters (that is 21.05% in females and 8.33% in males).

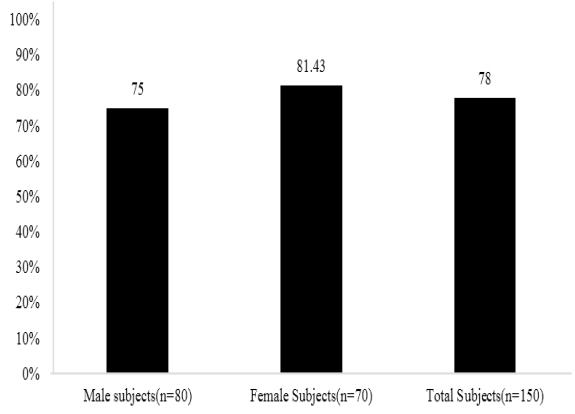

Figure 1. Prevalence of dyslipidemia in the subjects

The present study reports a high prevalence of dyslipidemia (78%) among apparently healthy urban obese adults. The study results agree with many other studies that highlight the substantial burden of dyslipidemia in India.

The overall prevalence of dyslipidemia in India ranges from 10% to 73% depending on the area of residence (rural vs. urban), socio-economic strata (high vs. middle low), diet and physical activity patterns and age (Ranganathan *et al.* 2015).

Numerous studies have documented the prevalence of various forms of lipid disorders among Indians. Not only is the prevalence of dyslipidemia high among Indians, but it has also steadily increased in recent decades. Adults who are obese also have a greater risk of establishing dyslipidemia and associated health problems. Recent research has shown that adults with severe obesity may have a lower life expectancy of 15–20 years than individuals who are normal weight. In obese adults, a large proportion of morbidity and mortality is attributed to sudden cardiac arrest and congestive heart failure which is associated with obesity (Chitra *et al.* 2012). Gupta *et al.* (2008) reported a significant association between increasing dyslipidemias with increasing obesity and truncal obesity

Indian dyslipidemic adults are often characterized by having significantly lower high-density lipoprotein cholesterol and elevated triglyceride levels (Dalaal *et al.* 2016). The same characteristics were reported in the present study where low HDL levels and high triglycerides were the most common abnormal lipid parameters. The ICMR–INDIAB study that was conducted in four Indian states (i.e., Maharashtra, Jharkhand, Tamil Nadu and Jharkhand) reported that despite the regional differences, low HDL-C was the most common lipid abnormality in all the four regions studied and 44.9% had isolated low HDL cholesterol (Joshi *et al.* 2014). This study thus confirms the findings of several earlier

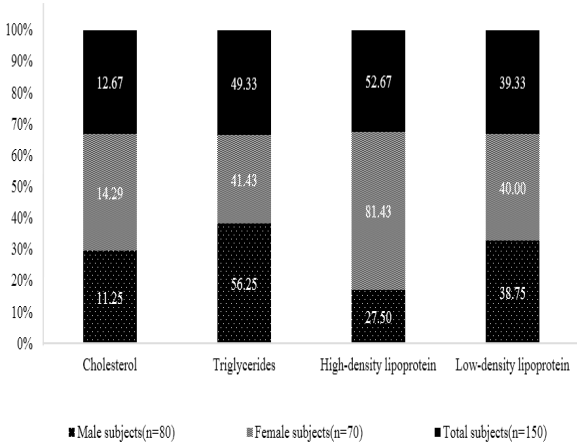

Figure 2. Distribution of subjects based on the abnomal lipid parameter

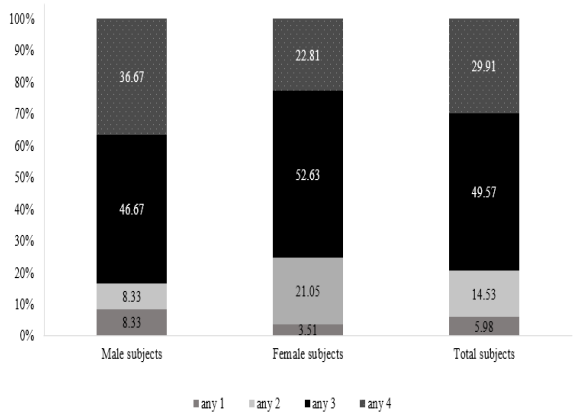

Figure 3. Distribution of the dyslipidemic subjects with number of abnormal blood-lipid parameters present by NCEP-ATP III Guidelines

studies that Indians have a high prevalence of low HDL cholesterol. This appears to be part of the Asian Indian phenotype (Deepa *et al.* 2006). Ranganathan *et al.* (2015) reported a high prevalence of dyslipidemia in the obese adults in comparison to adult with normal BMI in Salem, Tamil Nadu. Among the high BMI group, 59% had hypercholesterolemia, 42% had HDL-C of <30 mg/dl, 41% had LDL-C of >130 mg/dl, 56% had very-low-density lipoprotein (VLDL)-C of >40 mg/dl and 55% had TG >150 mg/ dl.

One of the characteristic features of obesity-related dyslipidemia is elevated fasting and postprandial TG combined with the prevalence of small dense LDL and lower levels of HDL-C (Klop *et al.* 2013), Indians and migrant from South Asia tend to have higher triglyceride levels and lower HDL cholesterol with lower total cholesterol levels than in the general population in the US and the UK (McKeigue *et al.* 1989; Enas 2000). This is in concordance with the present study as high cholesterol was the least prevalent abnormal lipid parameter among the subjects. Data from the western countries illustrates that total cholesterol is the most common deranged lipid parameter and unlike South Asians, they are reported to have better HDL levels. In recent research among Asian Indian immigrants in the United States, it was noted that the prevalence of hypercholesterolemia was 43.5%, while that of hypertriglyceridemia of 42.3%, low HDL and high LDH were 42.3%, 26.4% 41.4% respectively (Misra *et al.* 2010). Jordanian adults reported similar prevalence rates, with a prevalence of 48.8% hypercholesterolemia, 43.6% hypertriglyceridemia, 40.1% low HDL-C and 40.7% high LDL-C (Khader *et al.* 2010). Among Turkish adults also the prevalence rates were much similar that is, hypercholesterolemia (37.5%), high triglycerides (30.4%), low HDL-C (21.1.%) and high LDL-C (44.5%) (Erem *et al.* 2008).

CONCLUSION

The study result highlights the high prevalence of dyslipidemia (78%) among adult obese subjects who were apparently healthy. It was observed that low HDL levels and high triglycerides were the most common abnormal lipid parameters. The results are of great concern as the subjects had no prior history of any chronic diseases. The high prevalence among asymptomatic urban adults emphasizes the importance of regular and timely screening for apparently healthy populations and further advocating adequate medical and dietary intervention that can help in controlling and preventing health-related complications.

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formalism and drafted the manuscript. Both H.K and BTA contributed to the final version of the manuscript. BTA supervised the project.

AUTHOR DISCLOSURES

The authors have no conflict of interest.

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Prevalence and Determinants for Hypertension among Rural Women of Reproductive Age in Indonesia

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ABSTRACT

This study aimed to investigate the prevalence and determinants of hypertension in rural women of reproductive age. The cross-sectional study was conducted in Cianjur district on August 2019. It involved 193 married rural women aged 20–49 years old. Independent variables investigated were socio-economic characteristics, anthropometry, fat distribution and nutrient intake. The prevalence of hypertension in the study population using the new American Guideline (ACC/AHA) was 58% in contrast to 23.8% using the European (ESC/ESH) cut offs. Socioeconomic characteristics and nutrient intake showed no significant association with hypertension ($p>0.05$) while, Body Mass Index (BMI) ($p>0.012$), Visceral Fat (VF) ($p>0.013$) and Waist Circumference (WC) ($p>0.010$) were significantly associated with hypertension. Hence, result of binary logistic regression showed waist circumference is the strongest factor to determine hypertension in our study (OR=2.2; 95% CI: 1.20–4.01). Overall, the use of the American guideline increases the sensitivity of hypertension screening. The use of simple anthropometry measurement of WC combined with BMI can be applied for risk screening of hypertension in the primary health care setting including in the integrated community health post to improve preventive measure. Due to several limitations in the current study, future study should consider larger sample size and addresses history of hormonal contraceptive use as well as physical activity.

Keywords: hypertension, waist circumference, women

INTRODUCTION

The number of adults with hypertension has increased rapidly, where the low-income and middle-income countries are the greatest contributors (NCD-RisC 2017) Globally, high Systolic Blood Pressure (SBP) is the highest leading cause of DALY lost in female population, it accounted for 89,9 million DALYs; 80,9 million to 98,2 million in 1990 to 2016 (GBD 2017). At national level, the prevalence of high blood pressure among women aged ≥18 years old in Indonesia was 36.85% which is higher compared to the male population. Moreover the prevalence among farmers and those living in West Java were also high (MoH RI 2019a).

Older women as they aged and their estrogenic level is decreasing, are more prone to hypertension (Wenger *et al.* 2018). However, Hypertension is a chronic condition where the pathophysiology may rises long before the actual increase of blood pressure. Thus, in order to improve preventative measure screening of blood pressure and its risk among younger age group is pivotal. In addition, despite the relatively lower prevalence of hypertension in women of reproductive age, hypertension in women within this age group has its own specific clinical implications and challenges such as when the women with hypertension become pregnant. Women with chronic hypertension showed higher risk of adverse pregnancy outcomes such as preterm delivery, foetal growth restrictions as well as perinatal death amongst other (Bramham *et al.* 2014).

In Indonesia, around 44% of all female within the reproductive age of 20–49 years are living in rural area (BPS 2018). The rural and urban population are facing different risk factors, with the rural population often lack in health care access and utilization. In China, awareness and treatment control of hypertension were better in urban setting compared to the rural area, despite the similar prevalence (Li *et al.* 2017).

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In Indonesia, the welfare statistics also mirror the same insight, where only less than half of the rural women seek health care when they are sick (BPS 2018). Therefore, a study focusing on rural women of reproductive age will provide information on how to reduce the morbidity and mortality related to hypertension within this specific population.

Due to the importance of hypertension in the community, experts in this field issued guidelines on its management periodically. The latest European guidelines retain the previous definition of hypertension (BP>140/90 mmHg) (Williams *et al.* 2018). Whereas the new American guidelines lowered the threshold to define hypertension to <130/80mmHg (Goel *et al*. 2019) The lower cut off is important for two epidemiological reasons. The first is that high blood pressure is still the main risk factor for mortality and morbidity. Secondly, several metaanalyses indicate that the risk of coronary events and stroke among subjects with SBP between 130 and 139 is 1.5–2 times higher compared to those who have SBP of below 120 (Sierra 2019).

The same argument is also used by experts in India to advocate adoption the American College of Cardiology (ACC)/ American Heart Association (AHA) guidelines (Chopra & Ram 2019). On a larger context of Asian population with stroke as the dominant complication of hypertension not cardiac events, the use of relatively low diagnostic thresholds and therapeutic targets (130/80 mmHg), as suggested by the ACC/AHA Guidelines considered more acceptable (Angeli *et al.* 2019). Thus, acknowledging the need for aggressive BP goals and lower diagnostic threshold bot at clinical setting and screening purposes will provide enormous public health benefit for Asian population.

Indonesian women and farmers are among the most affected based on the recent national health survey data. However, to our knowledge previous study done regarding hypertension in rural adult population in Indonesia was focused on older age group, not specific in women and utilizing higher cut off value for diagnosis (Diana *et al.* 2018). Hence, due to paucity of research on hypertension among rural women of reproductive age utilizing the recent ACC/AHA guidelines, the study aimed to investigate the prevalence and determinants of hypertension in the target population.

METHODS

Design, location, and time

The cross-sectional study was conducted in Sukabungah and Campaka Mulya Villages, Cianjur district–West Java, Indonesia on August 2019.

Sampling

The minimum sample size was calculated using the Lameshow equation (Pourhoseingholi *et al.* 2013) with 95% CI, 10% precision and previous known prevalence for hypertension of 36.85% among Indonesian female population (MoH RI 2019a). The study involved 193 married rural women aged 20–49 years old, the exclusion criteria were pregnancy and any physical disability that prevent accurate measurement of anthropometry, body fat distribution or blood pressure. The ethical clearance for the study was issued by the Ethical Committee of IPB University in Bogor (Number 210/IT3.KEPMSM-IPB/ SK/2019).

Data collection

Blood pressure measurement. Blood pressure was measured in health facility in the morning (08.00–11.00 a.m.) with around 15 minute rest after the respondents were arriving at the facility. Measurement was done using an automatic blood pressure monitor on the left arm with a validated upper-arm-cuff device (OMRON-HEM7130). Respondent was in relaxed sitting position, legs not crossed, not talking, arm rested on the table level to the heart. Average of three measurements was taken, the three measurements were taken sequentially with more than one minute apart. On an important note, such measurement alone is not appropriate for the diagnosis of hypertension in untreated subjects, however it does have a role in screening for hypertension (Stergiou *et al.* 2018). Shortly following the blood pressure measurement, respondents were asked "Have you ever diagnosed with high blood pressure (hypertension)?" Those responding affirmatively, were then asked if they had taken any medication for high blood pressure in the last one month.

Socio-economic characteristics. In addition to age (date of birth), data regarding education level, income level, and occupation status were also obtained via interview using

a structured questionnaire. The interview for individual characteristics were done at home before the physical examination was performed parallel to the first 24 hours food recall.

Nutrient intake. The nutrients intake data was obtained from 2x24 hours food recall, first taken at home during a workday and second at the health facility during weekend. The food recall process was assisted with the national food photograph book to increase precision. Nutrient intake value were obtained from NutriSurvey® for Windows 2007 app, incorporating the nutrient from the Indonesian food data base. Nutrient intake obtained were nutrients related to Dietary Approaches to Stop Hypertension (DASH) diet. (Kim & Andrade 2016).

Anthropometry and fat distribution measurements. The anthropometry measurements were taken after the blood pressure measurement. Height, waist circumference, body weight, Body Mass Index (BMI), visceral fat and total body fat composition were performed. Body height was measured with a stadiometer with a capacity of 200 cm and an accuracy of 0.1 cm. The waist circumference was measured with a measuring tape with a capacity of 150 cm and an accuracy of 0.1 cm. The body weight, BMI and fat composition were measured with a four electrodes (two hands and two feet) Bioelectrical Impedance Analysis (BIA) monitor (OMRON® Karada Scan Body Composition Monitor HBF358-BW) in light clothing without shoes or socks on.

Data analysis

Hypertension was defined as mean Systolic Blood Pressure (SBP) ≥130 mmgHg or Diastolic Blood Pressure (DBP) ≤80 mmHg, following the new ACC/AHA guidelines (Sierra 2019).

Short stature was defined as height ≤152 cm (Ferreira *et al.* 2009). Respondents' age, education and income level were classified into three groups. For age the groups were 20–29 years, 30–39 years and 40–49 years. While for education the classification were elementary school or lower, Junior high school and senior high school or higher. The category for household income level were household income of under one million, one to two millions and above two millions rupiah per month, where 1,8 million rupiah per month is the minimum regional wage in the West Java province. Mothers were categorized into working mother and home maker.

The BMI category was based on the Asian cut off for overweight and obesity which is ≥23 kg/m^2 for elevated risk of non-communicable disease (WHO 2004). The visceral fat cut off measured by BIA to define metabolic syndrome in our study was 9% (Ozhan *et al.* 2012), as for the waist circumference we refer to the Asian female population of above 80 cm for increased risk of metabolic syndrome (WHO 2008). Regarding nutrient intake, the respondents' nutrients intake for each nutrient was categorized in two groups of lower risk and high risk. The cut off value for increased or higher risk of hypertension from each nutrient was based on the DASH diet cut off namely for saturated fat ≤6% of energy, total fat ≤27% of energy, protein ≥18% of energy, fibre ≥14.8 g/1,000 kcal and sodium ≤1,143 mg/1,000 kcal (Mellen *et al.* 2008).

All collected data in this report are first presented in a descriptive statistics. Further analysis of the associations between variables with the occurrence of hypertension as dependent variable were done using the Chi-square test ($p<0.05$). Binary logistic regression using the backward Wald method was performed to analyse the factors that determine hypertension involving all significant factors resulted from the Chi square test.

RESULTS AND DISCUSSION

Prevalence of hypertension

The prevalence of hypertension screened using the ACC/AHA guidelines in our study population was 58% or more than double the prevalence if measured using the ESC/ESH guideline which was only 23.8%. The large majority of the respondents (96.4%) have never diagnosed for hypertension. Among those who have ever been diagnosed with hypertension, none were currently taking any blood pressure medication during the past month. The average SBP in our study was 122.2 mmHg (SD 15.5mmHg) while for the DBP was 81.0 mmHg (SD 8.9 mmHg), this diastolic blood pressure is above the ACC/ AHA cut off for DBP (Sierra 2019).

Socio-economic characteristics

The average age was 31.15 (SD 6.84) years. Their education level were mostly low with

66.3% of them had an elementary school level education or lower and only 4.7% had a senior high school level education or higher. As for income level, more than half of our respondents were from the lower household's income group. The Chi square test did not show any significant association between soci economic characteristics with hypertension as shown in Table 1. However it is important to highlight that although it was not statistically significant, hypertension prevalence was observed the highest in oldest age group where 76.2% of women in the age of 40–49 had high blood pressure. In the U.S, women aged 35–44 years had a three times higher risk for hypertension compared to women aged 20–25 years old (Chen & Chauhan 2019). On an important note for our study method, is that the main pathophysiology for risk of hypertension in older women is related to the exposure to the sex hormone. Meta-analysis had shown certain duration of hormonal contraception such as the oral contraceptive use increases the risk for clinical hypertension (Liu *et al.* 2017). Therefore, the limitation of this study was to not take any history of hormonal contraceptive, which should be considered in future research.

In addition to age, more than half of the women from the two households' income brackets of lower than 2 million rupiah were suffering from hypertension compared to around 40% in the highest income level. Studies have shown conflicting evidence on the associations of income and hypertension among women in the developing countries. Studies from South Africa and India showed that women with higher socio economic level had lower risk of hypertension (Cois & Ehrlich 2014; Tyagi *et al.* 2015). But, in Mexico, increased socio-economic status positively correlated to the increase in SBP among low income women (Fernald & Adler 2008). Therefore, more robust methodology is needed to define the associations between income and hypertension.

Nutrient intake

Overall, the average intake of energy, protein, fat, fibre and sodium of our respondents were lower than the national RDI for nonpregnant woman aged 19–29 and 30–49 years old (MoH RI 2019b). The average intake for macro nutrients and micronutrients of interest are as follow: energy intake was 1,347 kcal (SD 458 kcal), Protein intake 40.6 g (SD 16.8 g), total fat intake 46.5 g (SD 23 g), fibre intake 5.8 g (SD 3 g), Saturated fat intake 21.3 g (12.4 g) and Sodium intake 629 mg (SD 447 mg)

The Chi square test showed no significant associations between nutrient-intake for protein, total fat, saturated fat, sodium and fibre according to the DASH nutrient cut off value presented in method (Mellen *et al.* 2008) with hypertension in our respondents (Table 2). Despite the non significant associations for protein and total fat intake, higher prevalence of hypertension were

Table 1. Chi square test for socio-economic characteristics

Independent variables	Normal blood pressure		Hypertension		p value
	n	Observed %	n	Observed %	
Age					
20–29	37	42.5%	50	57.5%	0.184
30–39	39	45.9%	46	54.1%	
40–49	5	23.8%	16	76.2%	
Education					
Elementary school or lower	54	42.2%	74	57.8%	0.931
Junior high school or higher	27	41.5%	38	58.5%	
Income					
<1 million	41	41.8%	57	58.2%	0.176
1–2 Million	26	36.6%	45	63.4%	
>2 million	14	58.3%	10	41.7%	
Occupation					
Home maker	20	48.8%	21	51.2%	0.319
Working	61	40.1%	91	59.9%	

Significant $^{*}p < 0.05$

found in respondents who consumed protein lower than 18% of the total energy and fat of higher than 27% of total energy intake. These findings are consistent to studies pointing out that protein is likely a protective factor for high blood pressure. (Elliott *et al.* 2006; Buendia *et al.* 2015) Recent study among 28,100 adult U.S women found that higher intake of Saturated Fatty Acids (SFAs), Mono Unsaturated Fatty Acids (MUFAs), and trans FAs was each associated with increased risk of hypertension among middle-aged and older women, whereas only association for trans Fatty Acids (FAs) remained statistically significant after adjustment for obesity-related factor (Wang *et al.* 2010).

We found no significant association between sodium intake and hypertension. Prolonged high sodium intake will cause increased sodium level in the cerebrospinal fluid, which activates the increasing sympathetic outflow and leading to hypertension. (Takahashi *et al.* 2011). The lack of association found within our study might related to the fact that the sodium intake of all our respondents was low.

In addition to protein, fibre intake is considered as a protective factor for hypertension. A Meta-analysis of Randomized Placebo Controlled Trials found that fibre supplementation reduced both systolic and diastolic BP (Streppel *et al.* 2005). While for the protective mechanism of fibre against hypertension several hypothesis were raised. The β-Glucan and other soluble fibre components have been proposed to have in particular hypo-cholesterolemic effects, but fibre can also improve glycaemia, insulin resistance, triglyceride levels and weight loss. These effects could probably lead to an improved cardiovascular condition thus resulted in better cardiac health and lower blood pressure (Aleixandre & Miguel 2016). Sadly, in our respondents the average intake of fibre was very low of around 20% from the national RDI for non-pregnant women of reproductive age and all were below the DASH cut off value. Hence, an education on the adequate amount of intake with easy to understand message and visual cue is pressing to improve consumption of fibre as protective factors for hypertension among this population.

Anthropometry and fat distribution

The average BMI of our respondents was 25.87 kg/m^2 (SD 4.2 kg/m^2) this is above the Asian cut off for higher risk of chronic non-communicable diseases of 23 cm (WHO 2004). The total body fat level was also above the normal 30% cut off the same goes for waist circumference in which the average was 85.61 cm (SD 24.7 cm)

Table 2. Chi square test for nutrient intake

Nutrient intake	Normal blood pressure		Hypertension		p value
	n	Observed %	n	Observed %	
Protein intake					
Equal to or higher than 18% of energy intake	3	60.0%	2	40.0%	0.41
Lower than 18% of energy intake	78	41.5%	110	58.5%	
Total fat intake					
Equal to or lower than 27% of energy intake	31	46.3%	36	53.7%	0.38
Higher than 27% of energy intake	50	39.7%	78	60.3%	
Saturated fat					
Equal to or lower than 6% of energy intake	45	41.7%	63	58.3%	0.92
Higher than 6% of energy intake	36	42.4%	49	57.6%	
Sodium					
Lower than or equal to 1,143mg/1,000kcal	75	41.9%	104	58.1%	0.94
Higher than 1,143mg/1,000kcal	6	42.9%	8	57.1%	
Fibre	Analysis for fibre intake were unable to be performed since the variable is a constant (all are below the 30 g cut off)				

Significant $^*p<0.05$

or above the 80 cm cut off (WHO 2008). This anthropometry measurement were in contrast to the total energy intake as recorded from the 24 hours food recall, thus under reporting of intake might have caused the discrepancy. On the other hand, the average visceral fat of our respondents were 7.53% (SD 4.2%) or lower than the 9% cut off applied in Egyptian population (Ozhan *et al.* 2012). The average height of our respondents was also low (14.8 cm; SD 4.7 cm) or below the 152 cm cut off for short stature.

The Chi-square test result showed that Body Mass Index (BMI) (p>0.012), Visceral Fat (p>0.013) and Waist Circumference (p>0.010) were significantly associated with hypertension. While for total body fat and height, the prevalence were higher among respondents with higher total body fat (61%) and shorter stature (61.3%). The Chi square test results are presented in Table 3. These result are in line with many studies on determinants of hypertension where, higher BMI were positively associated with both prehypertension and hypertension in adult (Khanam *et al.* 2015; Kibria *et al.* 2019; Chen & Chauhan 2019).

Associations between early growth and cardiovascular disease, first shown by Barker, have been found in many studies. Our result shows the prevalence of hypertension among shorter women (height<152 cm) was 61.3% compared to 49% among taller women. A study of Brazilian women found that short stature, independent of confounding by race and environmental factors, was an important risk factor for hypertension among women. (Sichieri *et al.* 2000). More recent study consistently found that Brazilian women of short stature presented a higher prevalence of chronic degenerative diseases (Ferreira *et al.* 2009). This finding might be used in future studies to identify factors related to the pathogenesis of hypertension linked to developmental and nutrition problems such as stunting.

Metanalysis study showed that the use of BMI alone to diagnose obesity despite of its specificity, provides low sensitivity to identify adiposity or excess body fat (Okorodudu *et al.* 2010). The association of waist circumference and hemodynamic has been long studied. In Indonesia, a case control study in Surabaya showed that Waist Circumference (WC) as the strongest determinant for hypertension in clinical setting (Mafaza *et al.* 2016). Consistently, a cross sectional study in rural population of older adult in Indonesia also showed the same result, where respondents with a waist circumference of above the normal cut off had a four times greater chance for hypertension (Diana *et al.* 2018).

Binary logistic regression analysis on variables significantly associated with hypertension namely, BMI, waist circumference

Table 3. Chi square test for anthropometric assessment

Variable	Normal blood pressure		Hypertension		p value
	n	Observed %	n	Observed %	
Body mass index					
<23	29	56.9%	22	43.1%	0.012*
≥23	52	36.6%	90	63.4%	
Height					
≥152	26	51.0%	25	49.0%	0.128
<152	55	38.7%	87	61.3%	
Visceral fat					
<9	61	48.4%	65	51.6%	0.013*
≥9	20	29.9%	47	70.1%	
Total body fat					
<30	26	50.0%	26	50.0%	0.170
≥30	55	39.0%	86	61.0%	
Waist circumference					
<80	37	54.4%	31	45.6%	0.010*
≥80	44	35.2%	81	64.8%	

Significant *p<0.05

and visceral fat categories showed that waist circumference is the strongest determinant for hypertension in our study population (Table 4). Respondents with a waist circumference of ≥80 cm had 2.2 times risk to suffer from hypertension (OR= 2.2; 95% CI: 1.20–4.01).

The Chi-square and binary logistic regression result, highlighted the importance of adiposity measured by Obesity (BMI), Central Obesity (WC) and Visceral Fat as risk factors for hypertension. The mechanisms by which obesity leads to hypertension is not completely understood. It was thought to be mediated by physical compression by fat around the kidney, followed by activation of the renin-angiotensinaldosterone (RAA) and increased in sympathetic nervous systems activity. These mechanism initially increase renal sodium reabsorption, impair renal-pressure natriuresis, and ultimately raise blood pressure (Hall *et al.* 2015). Other factors such as lipotoxicity and endothelial and vascular dysfunction may accompany and/or exacerbate increased blood pressure as obesity is sustained. Under physiological conditions, perivascular adipose tissue (PVAT) attenuates agonist-induced vasoconstriction by releasing vasoactive molecules. However, in obese subjects PVAT contributes to endothelial dysfunction, chronic low grade inflammation, vasoconstriction, sodium retention and, consequently, arterial hypertension (Virdis 2016).

Referring to our result where both BMI and WC are significantly associated with hypertension and only WC was a strong determinant for hypertension, it revokes the old debate whether WC should be measured in addition to or instead of BMI for obesity-related cardio metabolic risk assessment. Data from the INSPIRE ME IAA study provide additional evidence that, rather than replacing BMI as the preferred adiposity index, WC should be assessed in addition to the BMI because the combined use of both anthropometric measures allows for stratification of subjects according to their level of VAT (Nazare *et al.* 2015). The combination of BMI and WC is a good surrogate marker of visceral fat accumulation and is useful for monitoring the results of lifestyle changes.

Table 4. Binary logistic regression (backward wald)

Variable	B	p	OR (95% CI)
Constant	-1.77	0.467	
Waist circumference	0.787	0.010	2.20 (1.20–4.01)

Nagelkerke R Square=0.46

Non-pharmacologic interventions addressing concomitant risk factors, especially those directed at reducing central obesity (eg, caloric and sodium restriction, modest alcohol consumption, regular physical activity) are important for lowering BP and reducing vascular risk in hypertensive women (Geraci & Geraci 2013). Further, in Indonesian study the risk of obesity in adults can be reduced by not starting smoking at early age and reducing consumption of fatty foods (Sudikno *et al.* 2018). These life style changes and modification should be offered as early as possible once increase in BMI and/or WC are found to prevent onset of hypertension among women of reproductive age.

Several limitations need to be acknowledged, however, when interpreting the present findings. First, our study was restricted to reproductive-aged women of 20–49 years, which should not be extrapolated to women in other age group. Second, some study data rely on self reported information such as the food recall may be subject to recall and social desirability bias, thus we carefully utilized the food photograph book to guide our data collection procedure a more precise tools such as food models may improve the recall quality. Finally, our study design was cross-sectional, which is limited in establishing causality. In addition, the lack of data regarding the use of hormonal contraception and level of physical activity limit further analysis related to the respondents life style as well as calculation of individual energy need to explain the high average of BMI and WC. Hence, this study is able to offer understanding on the high prevalence of hypertension among rural women of younger cohort as well as its associated factors to design future study. Consequently, our study is also able to provide insight for designing appropriate hypertension screening and prevention method in the Indonesian rural community setting.

CONCLUSION

The use of the American guideline increases the sensitivity of hypertension screening. The

prevalence of hypertension screened using the ACC/AHA guidelines in our study population was more than double the prevalence if measured using the ESC/ESH guideline (58% vs 23.8%). Socioeconomic characteristics and nutrient intake showed no significant association with hypertension. Body Mass Index (BMI), Visceral Fat (VAT) and Waist Circumference (WC) were significantly associated with hypertension in Chi Square test. Hence, waist circumference is the strongest determinant for hypertension in our study, where respondents with a WC of above 80 cm have 2.2 times higer risk to be hypertensive. The use of simple anthropometry measurement of BMI combined with WC together with a routine blood pressure check can be applied for risk screening of hypertension among women of reproductive age in the primary health care setting, including in the integrated community health post during their children visit to improve health care service for women of reproductive age and early management of chronic hypertension. Life style changes and modification should be offered as early as possible once increase in WC and/or BMI in non-pregnant women are found, to prevent earlier onset of hypertension among women of reproductive age. Due to several limitations in the current study, future cross sectional study should consider larger sample size and addresses history of hormonal contraceptive use as well as physical activity. Longitudinal studies will be needed to substantiate evidence on causality of the determinants.

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AUTHOR DISCLOSURES

The authors have no conflict of interest.

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Prevalence of Masked Obesity Associated with Lifestyle-Related Habits, Dietary Habits, and Energy Metabolism in Japanese Young Women

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ABSTRACT

We investigated the prevalence of Masked Obesity (MO) and the correlations between MO and lifestyle-related habits (e.g., exercise habits, dieting habits), dietary habits, energy metabolism, and seasons. The subjects were 131 young Japanese college students. Body composition was measured by bioelectrical impedance method and Resting Metabolic Rate (RMR) was measured by an indirect calorimeter. Subjects with a BMI in the normal range (n=110) were divided into the MO (percentage of body fat to Body Weight [BF] ≥30%) and control (C) (BF<30%) groups. Dietary energy and nutrient intakes were calculated from weighed dietary records. A questionnaire on lifestyle habits was obtained individually from the subjects. The percentage of MO was 32% of subjects within normal BMI. The prevalence of MO was the highest in winter, probably due to accumulation of body fat as an adaptation to cold. The MO group had low Fat-Free Mass (FFM) and high BF. RMR of the MO group was significantly lower than that of the C group. The MO group tended to have poor exercise habits, more dieting (restricting calorie intake) experiences and consumed a diet with less vegetables and beans. We concluded that the prevalence of MO was 32%; it was the highest in winter for subjects who had high fat and low FFM. This fact may be due to poor exercise, more dieting experiences and insufficient intake of vegetables and beans. Furthermore, this accumulation of body fat may be partly due to low RMR.

Keywords: body mass index, energy metabolism, life-related habits, masked obesity, seasonal variation

INTRODUCTION

Body Mass Index (BMI: expressed as body weight in kilograms divided by height in meters squared, kg/m^2) is a useful tool for defining anthropometric characteristics and determining obesity. However, a disadvantage of BMI is that it does not reflect the precise levels of body fat. Recently, obesity has been found in young Japanese women whose BMI is in the normal range (18.5≤BMI<25 kg/m^2), but have a relatively high percentage of body fat based on Japanese standards. This phenomenon called "masked obesity" has been detected often (Takahashi *et al.* 2002), and its prevalence is growing. It has been suggested that masked obesity is associated with a higher risk of developing serum lipid abnormalities, arteriosclerosis, and obesity. Additionally, obesity at a young age is associated with high morbidity and mortality due to arteriosclerotic disease, even if obesity is improved in adulthood (Must *et al.*1992). Masked

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obesity is not easily detected on the basis of BMI alone; thus, body composition measurements are necessary for early detection of this condition (Oguri *et al.* 2006).

It has been speculated that masked obesity may be caused by young women consuming inappropriate diets and maintaining sedentary lives (Yoshiike *et al.* 2002). Yoshiike *et al.* found that the mean BMI of women aged 20 to 39 years in the National Health and Nutrition Examination Survey decreased between 1976 and 1995. In addition, the percentage of women in their twenties who were underweight (BMI<18.5 kg/m^2) was 13.4% in 1981; however, since then it increased continuously to 21.7% in 2016, with one in five young women being underweight (2017 National Health and Nutrition Examination Survey). Reports have suggested that increased thinness may be influenced by inappropriate dieting behaviors associated with young people's desire to be thin (Mase *et al.* 2012) and that these dieting habits may make small Fat-Free Mass (FFM) more likely to accumulate as body fat and influence the formation of masked obesity. This is because, in the case of normal body weight with low or normal body fat, energy restriction such as dieting will induce a greater loss of lean mass than body fat (Dulloo & Jacquet 1999).

Body composition and nutritional status (McKinney *et al.* 2008) change seasonally, and body weight, fat mass, and fat percentage are known to increase during the winter and decrease during the summer in young Japanese women (Yumigeta *et al.* 2015). Subcutaneous fat gain in winter (Ishigure *et al.* 1980) is an important adaptation to the cold environment (Glaser & Shephard 1963). It is unclear, however, whether seasonal changes in body fat are associated with the prevalence and development or improvement of masked obesity.

This study aimed to examine the prevalence of masked obesity and to comprehensively assess whether masked obesity is associated with lifestyle-related habits, eating habits, energy metabolism, and seasons.

METHODS

Design, location, and time

Participants were 131 female students in the fourth year who belong to the Faculty of Home Economics at a university in Kobe and live in the Kansai area (south-western half of Japan including Kobe or Osaka city). The study was conducted in the spring of 2015 and 2016. Twenty five subjects participated in a one year study investigating seasonal changes in body fat percentage in winter, spring, summer, and autumn of 2015–2016. Seasonal variation in the prevalence of masked obesity was examined from the body fat percentage. Informed consent was obtained in advance by explaining the aim and method of the study. This study was approved by the Human Ethics Research Committee of Kobe Women's University.

Sampling

Of the total participants, 14 students (10.7%) had a BMI<18.5 kg/m^2, and 7 students (5.3%) had a BMI≥25 kg/m^2. Data from these students (n=21) were excluded, thus data from the subjects with a BMI in normal range (n=110) were extracted for the study.

Definition of masked obesity and the categories

Masked obesity was defined as BMI (18.5≤BMI<25 kg/m^2) with percentage of body fat of ≥30% (Fukuoka *et al.* 2012). Subjects with a BMI in the normal range were divided into two groups: the masked obesity (MO: body fat ≥30%) group and the control (C: body fat <30%) group.

Data collection

Body composition was measured by Inbody 720 (Biospace, Tokyo, Japan) using the bioelectrical impedance method. The measurements by the instrument are highly correlated with the underwater weighing method and Dual-energy X-ray absorptiometry (DEXA) methods (Utter & Lambeth 2010; Cha *et al.* 1995; Malavolti *et al.* 2003). Therefore, the body composition analyzer was used for these measurements.

Body weight and body composition were measured for three consecutive days during the low basal body temperature period, immediately after the end of menstruation for each subject, and the mean value was used for the data. Measurements were performed under the following conditions: 3 hours after breakfast, after urinary and fecal excretion, and with changing into an examination suit.

Resting metabolic rate was measured at the same time as body composition, during the low

basal body temperature phase, to avoid the effect of sex hormones. Measurements were performed using a metabolic analyzer (MedGem+, MP Japan Co. Ltd.) while subjects were sitting in chairs and resting for about 10 min.

Dietary surveys were conducted on three consecutive days during the low basal temperature period (along with body composition measurements), using the self-weighing records method. Subjects were asked to use their dietary record forms and leaflets to document all of the ingredients and seasonings used as well as their weights. For the marketed products, the trade name, brand name, and amount of energy and nutrients displayed were recorded. Nutrition calculations were performed using Excel Eiyou-kun Ver.6.0 (Kenpakusha, Tokyo, Japan), and energy/nutrient intake per day was calculated.

A questionnaire survey was conducted to investigate lifestyle and dietary habits associated with masked obesity. Questionnaire items were related to dieting experience, and past and current exercise habits. Questionnaires were distributed at the time of body composition measurement; they were self-completed and collected on the same day.

Data analysis

IBM SPSS, Ver. 21 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. An unpaired t-test was used for testing between the two groups, a chi-square test was used for cross-tabulation, and a Pearson test for correlation. The level of significance was less than 5%.

RESULTS AND DISCUSSION

Physique of the subjects and prevalence of masked obesity

The subjects with a BMI in normal range (n=110) were extracted from the participants (n=131). Their height, weight, BMI, body fat, and fat-free mass are shown in Table 1. The height and weight of the subjects were within the range of body sizes in the results of the National Health and Nutrition Examination Survey in Japan (MoHWL 2017) aged 20–29 years (Table 1). Thus, the BMI of the subjects was 21.1±1.5 kg/m^2, which was similar to the BMI (20.6±3.3 kg/m^2) of the National Survey data in Japan (MoHWL 2017).

Among the subjects with a normal BMI, the prevalence of masked obesity (% body fat ≥30%) was about 32% in the spring where the experiment started (Figure 1). The prevalence of MO in our study was higher than that of MO reported in 2012 (17.8%) (Fukuoka *et al.* 2012) but is almost equivalent to that reported in 2017 (Takeda *et al.* 2017). The frequency of masked obesity seems to increase year by year. As masked

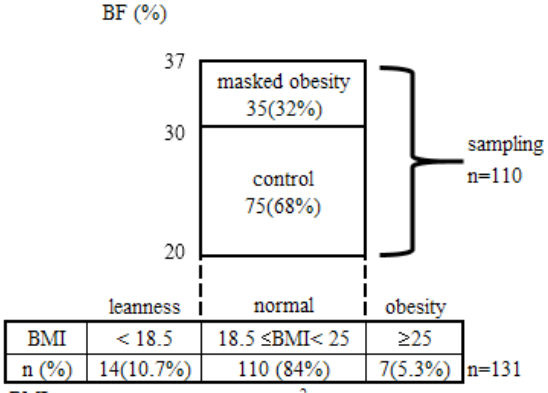

Figure 1. BMI distribution in participants(n=131) and classification of the subjects with a normal range of BMI by % body fat

Table 1. Physical characteristics of subjects

Anthropometric parameters	Subjects	Value	
		Minimum	Maximum
Height (cm)	158.1±5.0	146	169.4
Weight (kg)	52.8±5.1	42.3	66.3
BMI (kg/m^2)	21.1±1.5	18.5	24.7
Muscle mass (kg)	35.38±3.24	28.8	44
Skeletal muscle mass (kg)	20.43±2.08	16.1	25.8
Fat-free mass (kg)	37.81±3.45	30.7	47
Body fat mass (kg)	14.96±3.00	9.7	24.1
Body fat percentage (%)	28.21±3.93	19.57	37.1
Body water content (l)	27.72±2.55	22.5	34.3
Intra cellular water content (l)	17.20±1.59	13.9	21.4
Extra cellular water content (l)	10.49±0.93	8.6	12.9
Protein weight (kg)	7.44±0.69	6.0	9.2
Mineral amount (kg)	2.69±0.25	2.26	3.48
Waist hip ratio	0.81±0.29	0.75	0.88
RMR (kcal/day)	1,039±120	780	1,330

Values are means±Standard deviation (SD) (n=110); RMR: Resting Metabolic Rate; BMI: Body Mass Index
The subject with a normal range of BMI (18.5≤BMI<25) were extracted in the study

obesity cannot be detected without measuring body composition, some people with MO may remain unaware that their health is at risk. Hence, obesity awareness is important for preventing the development of obesity (Akil & Top 2019).

Comparison of body composition between masked obesity and normal individuals

Body compositions and resting metabolic rate of the MO group were compared with those of the C group (Table 2).

There was no significant difference between the MO and C groups in height, but body weight was significantly greater by 3.1 kg in the MO group than in the control. The BMI (=22 kg/m^2) in the MO group was also significantly greater than that (=20 kg/m^2) in the C group (Table 2); thus, the BMI in the MO group might be an indicator of masked obesity. In other words, a BMI of 22 in young Japanese women might imply a health risk instead of an appropriate BMI based on the World Health Organization standards (WHO expert consultation 2004). Thus, these results supported a WHO report that Asian populations might have risk factors for diabetes mellitus and cardiovascular disease even below a BMI of 25 kg/m^2 because Asian people generally have a higher body fat than white people of the same BMI(WHO expert consultation 2004).

Comparison of body composition showed that FFM was lower in the MO group by 1.4 kg than in the C group ($p=0.056$), and the body fat mass was significantly higher by 4.5 kg in the MO than in the control group ($p<0.001$). Thus,

Table 2. Comparison of body composition and resting metabolic rate between masked obesity group and control group

Body composition	MO (n=35)	C (n=75)	p value
Height (cm)	157.0±4.9	158.5±5.0	0.144
Weight (kg)	54.9±5.0	51.8±4.9	0.002
BMI (kg/m^2)	22.24±1.26	20.57±1.34	<0.001
Muscle mass (kg)	34.70±2.82	35.98±3.36	0.054
Skeletal muscle mass (kg)	19.85±1.76	20.69±2.17	0.049
FFM (kg)	36.89±3.00	38.24±3.59	0.056
Body fat mass (kg)	18.03±2.31	13.53±2.06	<0.001
Body fat percentage (%)	32.74±1.84	26.09±2.63	<0.001
Right arm muscle mass (kg)	1.65±0.21	1.70±0.24	0.351
Left arm muscle mass (kg)	1.62±0.20	1.65±0.25	0.562
Trunk muscle mass (kg)	16.15±1.38	16.33±1.59	0.556
Right leg muscle mass (kg)	5.75±0.64	5.96±0.69	0.133
Left leg muscle mass (kg)	5.76±0.63	5.96±0.68	0.131
Right arm fat mass (kg)	1.22±0.18	0.87±0.14	<0.001
Left arm fat mass (kg)	1.24±0.19	0.90±0.14	<0.001
Trunk fat mass (kg)	8.60±1.23	6.22±1.16	<0.001
Right leg fat mass (kg)	2.99±0.36	2.31±0.31	<0.001
Left leg fat mass(kg)	2.98±0.36	2.30±0.30	<0.001
Waist hip ration	0.83±0.03	0.81±0.03	<0.001
BMR (kcal)	1,166±65	1,196±77	0.056
RMR (kcal/kg body weight)	18.93±1.95	20.19±2.43	0.008

Values are means±SD (n=110); Unpaired t test; MO: Masked Obesity group; C: Control group
FFM: Fat-Free Mass; BMR: Basal Metabolic Rate; RMR: Resting Metabolic Rate

we found that the weight gain of masked obesity was due to an increase in body fat that greatly exceeded the decline in fat-free mass.

When comparing body parts, muscle mass in the trunk, and upper and lower limbs, the MO group was almost the same as that of the C group. On the other hand, body fat mass was significantly greater ($p<0.001$) in the MO group than in the C group in all body parts, including the trunk, upper limbs, and lower limbs. Approximately 30%–40% of fat in the trunk and the upper and lower limbs accumulated more than in the C group.

Resting metabolic rate was significantly lower in the MO group than in the C group. Lower energy metabolism in MO might easily promote fat accumulation.

Energy and nutrient intakes

Energy intake of the MO group was similar to that of C group (Table 3). Intakes of protein, fat, and carbohydrates in the MO group were also similar to those in C group. Similarly, the energy composition ratio of fat to total energy in the MO group was not different from that in the

Table 3. Composition of total energy intake (per day) and nutrient intakes (per day) of between masked obesity group and control group

Energy and nutrient intakes	MO (n=35)	C (n=75)	p value
Energy (kcal)	1,434±320	1,471±298	0.55
Protein (g)	53.41±13.60	55.87±14.66	0.40
Fat (g)	50.27±15.62	49.85±15.45	0.90
Carbohydrate (g)	191.61±52.81	191.04±45.49	0.95
Protein (%E)	14.93±2.09	15.13±2.41	0.67
Fat (%E)	31.42±6.42	30.35±6.63	0.43
Carbohydrate (%E)	53.50±10.00	52.18±7.79	0.45
Dietary fiber (g)	7.38±2.94	9.01±4.71	0.06
Sodium (mg)	2384.6±668.3	2495.9±776.8	0.47
Potassium (mg)	1689.1±662.2	1737.0±710.2	0.74
Calcium (mg)	292.8±128.4	347.8±153.8	0.07
Magnesium (mg)	145.5±42.7	175.3±72.9	0.01
Phosphorus (mg)	666.2±216.7	718.4±241.1	0.28
Iron (mg)	4.21±1.65	5.16±2.63	0.05
Zinc (mg)	5.51±2.08	5.97±1.92	0.26
Copper (mg)	0.66±0.21	0.76±0.26	0.07
Vitamin A (µg)	325.9±216.6	367.4±254.1	0.41
Vitamin D(µg)	4.12±3.09	3.63±3.38	0.47
Vitamin E (mg)	4.28±1.94	4.64±2.24	0.41
Vitamin K (µg)	123.6±73.67	168.4±251.2	0.31
Vitamin B1 (mg)	0.67±0.31	0.67±0.26	0.99
Vitamin B2 (mg)	0.82±0.29	0.86±0.33	0.52
Niacin (mg)	19.07±6.62	21.61±8.15	0.11
Vitamin B6 (mg)	0.81±0.30	0.89±0.35	0.22
Vitamin B12 (µg)	3.27±1.93	3.62±3.42	0.58
Pantothenic acid (mg)	4.06±1.46	4.28±1.52	0.48
Biotin (µg)	21.64±8.09	26.59±12.85	0.02
Vitamin C (mg)	90.27±113.5	71.12±44.37	0.34
Salt (g)	6.10±1.79	6.32±2.03	0.58

Values are means±SD (n=110); Unpaired *t* test; MO:Masked Obesity group
C:Control group; Protein (%E): The energy composition ratio of protein to total energy; Fat (%E): The energy composition ration of fat to total energy; Carbohydrate (%E): The energy composition of carbohydrate to total energy

C group. The energy intake in our data was lower by 200 kcal than the results presented by the National Health and Nutrition Survey in Japan, although it was not strictly comparable because of the different methods used (MoHWL 2017).

On the other hand, the intake of dietary fiber was lower in the MO group than in the C group by 18%, although there was insignificance (p=0.06) between them. Low dietary fiber intake might cause accumulation of fat in the MO group with the same energy intake as the control group (Table 3).

There were no differences between the two groups in sodium and potassium intakes; however, calcium, iron, and copper intakes tended to be lower in the MO group than in the control,

and magnesium intakes were significantly higher in the MO group than in the control. With respect to vitamins, the MO group tended to have less vitamins, especially significantly less biotin.

Relationship between body fat percentage and food intake

The correlation between the percentage of body fat and intakes of food groups such as vegetables or beans (Table 4) were examined in all subjects. There were significant and negative correlations between body fat percentage and intakes of vegetables and beans, respectively; a higher body fat percentage was associated with lower intakes of vegetables and beans.

There are many research studies on the correlation between the intakes of vegetables and fruits and the percentage of body fat. In Japan, the subjects with masked obesity consumed a smaller daily intake of green and yellow vegetables and light-colored vegetables, and they consumed fried foods more frequently than those with standard proportions (Takeda *et al.* 2017). In Canada, surveys of Atlantic areas where obesity frequently emerged suggested that lower consumption of vegetables and fruits was inversely associated with visceral fat, and higher consumption of vegetables and fruits was associated with lower body fat mass (Yu *et al.* 2017). In a systematic review, increased vegetable consumption caused weight loss and was associated with reduced risks of becoming overweight and obese (Nour *et al.* 2018).

There was also a significant negative correlation between soy intake and body fat percentage, indicating that higher soy intake was associated with lower body fat percentage (Table 4). This suggests that soy protein contains peptides that reduce body fat. Experimentally, it has been shown that consumption of soy protein reduces body weight and body fat mass in addition to lowering plasma cholesterol and triglycerides in humans and rats (Velasquez *et al.* 2007). Thus, the lower body fat percentage may be due to the body fat-lowering effect of soybean protein when bean intake was high.

Table 4. Correlation between body fat percentage and intake of vegetables and beans

Food group	Body fat percentage		
	n	r	p
Vegetables	131	-0.17	0.049
Beans*	131	-0.22	0.012

*Tofu, natto, atsuage, etc
Pearson

Therefore, the results suggest that unhealthy dietary habits such as the low intake of vegetables and soybeans might accelerate body fat accumulation and then lead to formation of masked obesity.

Comparison of exercising habits and dieting experiences between the MO and C groups

In lifestyle habits, 31.4% and 56% of the MO and C groups, respectively, reported having present exercise habit (Table 5). However, there was no difference between the MO and C groups in their past exercise habit. Accumulation of body fat may be attributable to excessive energy

Table 5. The percentage of the subjects with dieting experiences, present exercise habit and past exercise habit in masked obesity groups and control groups

Dieting experiences and exercise habit	MO n(%)	C n(%)	Total n(%)	p value
Dieting experiences				
Yes	27(77.1%)	34(45.3%)	61(55.5%)	<0.01
No	8(22.9%)	41(54.7%)	49(44.5%)	
Present exercise habit				
Yes	11(31.4%)	42(56.0%)	53(48.2%)	0.02
No	24(68.6%)	33(44.0%)	57(51.8%)	
Past exercise habit				
Yes	31(88.6%)	62(82.7%)	93 (84.5%)	0.43
No	4(11.4%)	13(17.3%)	17(15.5%)	

MO: Masked Obesity group; C: Control group

intake or physical inactivity (Weinsier *et al.* 2002). As energy intake of the MO group was almost the same as that of the C group (Table 3), less physical activity may easily lead to masked obesity (Table 5).

Dieting behaviors for thinness are thought to contribute to masked obesity (Mase *et al.* 2012). In the present study, 77% of the subjects in the MO group had dieting experiences, which was much higher than the 45% in the C group. Therefore, inappropriate dieting behaviors would be a risk factor for masked obesity in young persons with normal BMI because dieting in people with a normal physique might induce a greater loss of fat-free mass rather than fat (Dulloo & Jacquet 1999).

Physical inactivity was also strongly associated with FFM. FFM of the MO group was somewhat lower compared with that of the C group. Since muscle, the main part of the fat-free mass, is the largest tissue for burning fat, the decline in fat-free mass results in a substantial tissue reduction that burns fat (Dulloo&Jacquet 1999).

Seasonal changes in the prevalence of masked obesity

The prevalence of masked obesity was 48% in winter, 36% in spring and summer, and 40% in autumn (Figure 2). There were seasonal changes in the prevalence of masked obesity with the highest frequency in winter and the lowest frequency in spring and summer.

High body fat in winter is in accordance with the physiological mechanisms that adapt to cold by increasing the amount of subcutaneous fat in winter (Ishigure *et al.* 1980) (Glaser & Shephard 1963), suggesting that seasonal changes need to be considered and investigated in studies of masked obesity.

Strengths and limitations

Seasonal changes in the prevalence of MO have never been examined in previous studies; we addressed this gap and found the highest prevalence of MO in winter, with most subjects likely to be associated with poor exercise, inappropriate dieting experiences, and unhealthy dietary habits. Exercise and healthy food intake reduce accumulation of body fat, thus preventing MO. Therefore, measurement of body fat mass in body composition is essential for enhancing awareness about MO among those with normal body weight.

This study, however, has some limitations as well. First, the subjects do not include men. Second, the subjects were all in the narrow age range of 21 to 22 years. Third, the participants

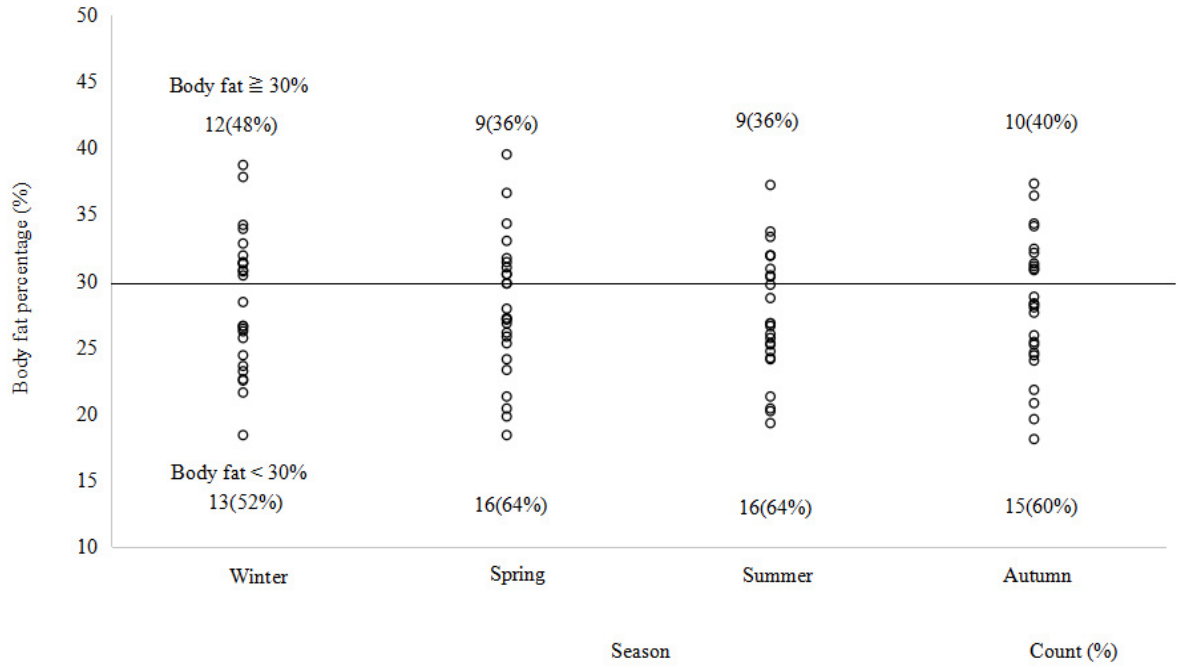

Figure 2. Seasonal changes in prevalence of masked obesity

were all (a) educated, (b) versed in the field of home economics, (c) women in a university, and (d) lived in urban areas. Thus, the results on a single population may not necessarily reflect physical condition of young women in general.

CONCLUSION

Masked obesity was found in about 32% of young women with normal BMI values in our spring study. There were seasonal changes in the prevalence of masked obesity with the highest frequency in winter and the lowest frequency in spring and summer. It was shown that the body composition of subjects with masked obesity consisted of low levels of fat-free mass and high levels of body fat, accumulated widely in the trunk, upper limbs, and lower limbs. Dietary habits with low vegetable and soy protein intake may accelerate body fat accumulation. Additionally, it was also speculated that the low FFM was due to inappropriate dieting and less exercising. Studies of masked obesity need to also consider seasonal changes.

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AUTHOR DISCLOSURES

The authors have no conflict of interest.

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Socioeconomic Characteristics, Nutritional Status, Health Status, and Quality of Life among Tea Plantation Workers in Pangalengan, West Java, Indonesia

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ABSTRACT

This cross-sectional study aimed to analyze the association between socioeconomic characteristics, nutritional status, anemia status and health status with the quality of life of tea pickers in Pangalengan, West Java, Indonesia. Subjects were 116 women of childbearing age (15–49) years. Anemia status data (hemoglobin levels) were taken using HemoCue Hb 201+, while nutritional status data were collected through anthropometric measurements (weight and height). Quality of life data was collected using the Short Form-36 (SF-36) questionnaire consisting of the Physical Component Summary (PCS) score and Mental Component Summary (MCS) score. PCS consists of physical function, physical role, pain, and general health dimensions. MCS consists of vitality, social functions, the role of emotions, and mental health. Data were analyzed using a Spearman correlation test, Pearson correlation test, and logistic regression test. The results showed that there was no significant relationship between anemia status and nutritional status with quality of life ($p>0.05$). However, non-anemic subjects tend to have higher PCS and MCS scores than anemic subjects. There was a positive relationship between Acute Respiratory Infection (ARI) with MCS in terms of social function dimensions, and joint pain with MCS in the emotional role dimension ($p<0.05$). The variables related to the quality of life were the number of family members and expenditures. Subjects with large family size (≥4 people) had a 3.5 times risk for experiencing lower quality of life compared with subjects with smaller family (<4 people) (OR=3.52; 95% CI:1.23–10.05). Subjects with monthly expense of >Rp. 343,646 had lower risk of experiencing low quality of life 59.7% compared to subjects who had household an expense of <Rp. 343,646 (OR=0.403; 95% CI:0.17–0.96).

Keywords: anemia, health status, nutritional status, quality of life, tea picker

INTRODUCTION

More than 30% of the world's population is anemic, but anemia is a difficult nutritional problem to solve. Most anemia is caused by iron deficiency and, in some areas, is exacerbated by the presence of infectious diseases (WHO 2008). The prevalence of anemia in Indonesia has not shown any significant decrease. The national Basic Health Research (Riskesdas) 2013 data shows the prevalence of anemia in women of childbearing age, was categorized as mild to moderate category of public health problem, which is 18.4–20.1% (Balitbangkes 2013).

Women of childbearing age are prone to experience iron deficiency anemia Iron Deficiency Anemia (IDA). During this reproductive age, iron deficiency and anemia can reduce work capacity or maximal aerobic capacity (VO2 max) and also cause a decrease in work productivity through decreased oxygen supply to the tissues (Haas & Brownlie 2001). The main symptom of anemia is fatigue. These symptoms can develop into other problems such as dizziness, depression, cognitive impairment, and others. The symptoms experienced often affect the quality of relationship and social roles due to lack of energy and interest in socializing (Cella & Breitbart 2001). In addition, there is a strong relationship between fatigue with physical health dimensions and emotional roles in the quality of life that can limit the subject at work or in daily activities (Efficace *et al.* 2016).

According to Fayers and Machin (2007), quality of life is a component of happiness and satisfaction with life. The definition of quality of

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life is often of a different meaning in each person because it has many influencing factors such as finance, safety, and health. For this reason, a term of quality of life-related to health is used in the field of health. The definition of health, according to the World Health Agency (WHO), is a state of well-being that includes physical, mental, and social, which is not only free from disease or disability. This means that the quality of human life related to health covers not only physical health, but also includes mental, social, and emotional health (Niswah 2014).

Health-related quality of life can decrease due to reduced functional abilities such as the ability to work, social interaction, recreational activities, and the decline in the meaning of subjective well-being (Flechtner & Bottomley 2003). Also, quality of life is affected by one's health status. Research by Zubaran *et al.* 2008 in 120 subjects in Brazil showed that there was a significant relationship between health status and quality of life. Health-related quality of life is also influenced by a person's nutritional status. Poor nutritional status can reduce physiological function, increase the risk of disease complications, and even death, which can result in decreased quality of life (Wanden-Berghe *et al.* 2009; Pearson *et al.* 2001).

Tea pickers are often the choice of work for women in rural areas. This is due to limited employment in rural areas, lack of skills, and low education (Kusumawati 2012). Tea pickers often find it challenging to obtain a healthy and proper settlement environment that affects the nutritional and health status, which can trigger various diseases (Fitriyani *et al.* 2008). This study aims to analyze the association between socioeconomic characteristics, nutritional status, anemia status, health status, with quality of life of tea pickers in Nusantara Plantation Company VIII (PTPN VIII) Pangalengan, West Java.

METHOD

Design, location, and time

The design of this study was cross-sectional. The research location was determined purposively, it was at the Nusantara Plantation Company VIII (PTPN VIII) located in Pangalengan, West Java, Indonesia. There are four selected gardens, namely Malabar Gardens, Purbasari Gardens, Sedep Gardens, and Talun Santosa Gardens. The study was conducted in April–July 2016. This research is part of a larger study entitled "Income Contribution, Food Consumption, Iron Deficiency Anemia among Women Workers in Tea Plantation and the Effect of Multinutrients Supplementation with Nutrition Education to Increase Their Productivity " chaired by Faisal Anwar.

Sampling

Subjects in this study were tea pickers. Inclusion criteria in the selection of subjects were women of childbearing age 15–49 years old, married/never married, not pregnant and/or breastfeeding, and were willing to participate in this study by filling out the informed consent. The minimum number of subjects was 116 people. This amount was obtained based on the formula from Lemeshow *et al.* (1997) by using the prevalence of anemia in women of childbearing age in West Java of 13.4% (BPPK Depkes RI 2008), the total population of 250, and the degree of trust of 5%. This research has received ethical approval from Diponegoro University No. 22/ EC / FKM / 2015.

Data collection

The type of data collected is primary and secondary data. The data in this study are baseline data from the leading research. Primary data include Haemoglobin (Hb) level, socioeconomic characteristics (age, marital status, household size, recent education, household expenses), health status, nutritional status (anthropometry and anemia status), and quality of life. Blood drawn for the Hb test did not require the subject to fast, the blood samples were analyzed using HemoCue Hb 201+. Data on subject characteristics and health status were obtained through interviews using a questionnaire. Quality of life data was collected through interviews with the Short Form-36 (SF-36) questionnaire consisting of the Physical Component Summary (PCS) and Mental Component Summary (MCS). PCS consists of physical function, physical role, pain, and general health dimensions. MCS consists of vitality, social functions, the role of emotions, and mental health. The SF-36 used has been translated and tested to have good internal consistency with an alpha coefficient ≥0.70 (Rachmawati *et al.* 2014).

Data analysis

The data was processed and analyzed using Microsoft Excel 2013 software and SPSS version 16.0. Subjects were categorized to be anemic if the Hb concentration was <12.0 g/dl. The calculation of nutritional adequacy level is calculated according to nutritional adequacy recommended by the Ministry of Health of the Republic of Indonesia (MoHRI 2013). The nutritional status classified as underweight (BMI <18.5 kg/m^2), normal (BMI 18.5–24.9 kg/m^2), overweight (25.0–26.9 kg/m^2), and obese (BMI> 27 kg/m^2). Health status data consists of a history of illnesses and health conditions within the past four weeks.

Questions contained in the SF-36 questionnaire (RAND 2009) were related to the subject's general perception of their health at the point of interview compared to four weeks ago. The SF-36 questionnaire consisted of 36 questions representing eight dimensions namely physical function (10 questions), physical role (4 questions), pain (2 questions), general health (5 questions), social function (2 questions), vitality (4 questions), the role of emotions (3 questions), and mental health (5 questions). Quality of life is categorized based on the average quality of life score. Subjects had a good quality of life if the score ≥74 and less if the score <74. The bivariate analysis uses the Pearson correlation test if the data is normally distributed and the Spearman Rank correlation test if the data is not normally distributed. To analyze factors affecting the quality of life, logistic regression analysis was used. The variables included in the logistic regression analysis were the variables which in the bivariate analysis have p<0.25. The results were said to be statistically significant when p<0.05.

RESULTS AND DISCUSSION

Socioeconomic characteristics

Table 1 shows that the mean age of the subjects was 41.7±5.5 years. More than half of the subjects (67.2%) were middle aged (41 to 60 years). According to Leslie & Hankey (2015), the amount of energy needed by the body will increase with age and reach its peak in adulthood, then the amount of energy needed by the body will decrease again afterward. In addition to decresing energy need, towards the age of 45 years, there will also be a decrease in work capacity, which includes functional, mental, and social capacity (McPhee *et al.* 2016).

Most subjects completed the elementary school level education (57.8%). According to Pradono and Sulistyowati (2014), low levels of education will affect the low ability to develop effective life capacities, which will ultimately affect the ability and skills to work, access to health facilities, welfare, and social support.

Almost all subjects had a small number of family members, less than four people (74.1%). The family size largely determines the family needs. More family members mean more resources are needed to meet the family needs (Erwin & Karmini 2012). The average expenditure per capita of the subjects was Rp. 626,958±Rp. 312,655. Most subjects were classified as not poor (82.8%). Almost all subjects were married (94.0%).

Table 1. Distribution of subject characteristics

Characteristics	n	%
Age (years)		
Young adults (18–40)	38	32.8
Middle adulthood (41–60)	78	67.2
Average±SD	41.7±5.5	
Education		
No school	1	0.9
Not completed in elementary school	35	30.1
Elementary school/equivalent	67	57.8
Junior high school/equivalent	12	10.3
High school/equivalent	1	0.9
Number of family members (people)		
A small family (≤4)	86	74.1
Medium family (5–6)	27	23.3
Large family (≥7)	3	2.6
Expenditures per capita		
Poor (<Rp. 344,000)	20	17.2
Not poor (≥Rp. 344,000)	96	82.8
Average±SD	626,958±312,655	
Marital status		
Married	109	94.0
Divorced	7	6.0

Nutritional status. Nutritional status was assessed through anthropometric methods (Body Mass Index) and biochemistry (hemoglobin levels). Table 2 shows that 44.0% of the subjects were classified as having normal nutritional status, but 53.4% of the subjects were classified as overweight and obese. The prevalence of subjects who had a BMI >25 in this study was greater than the Riskesdas data (2013), which was 32.9% and the research of Mahardikawati *et al.* (2008) on tea pickers in Pangalengan which was 30.4%. Good nutritional status is needed by tea pickers to be able to improve performance and productivity; this is mainly due to the work of tea pickers relying heavily on physical fitness (Kundu *et al.* 2013).

Anemia is a condition when the body lacks in red blood cells, or the concentration of hemoglobin in the blood is insufficient so that there is a disruption in transporting oxygen throughout the body (Balitbangkes 2013). Table 2 shows that the prevalence of anemia was 28.5%. The average hemoglobin level was 12.6 g/dl, with the lowest level was 9.1 g/dl, and the highest was 14.8 g/dl. The prevalence of anemia in this study is greater than the prevalence of anemia in women in Indonesia, which is 23.9%. Iron deficiency and anemia can reduce work capacity and productivity through decreased oxygen supply to tissues (Haas & Brownlie 2001).

Health status. Health status is a description of the history of the disease suffered by the subject at the point of interview and the subject's health condition in the last four weeks. Health is a basic right of every human being and is one of the factors that determine the quality of human resources. Table 2 shows that most of the subjects complained about Acute Respiratory Infections (ARI) (81.9%) and joint pain (73.3%) at the point of interview. Tea pickers work by collecting tea using a container that is carried on the back. The tea loads that reach about 40–75 kg/day on their back ca uses muscle tension and discomfort. Meanwhile, for the history of illness the majority (83.6%) of subjects had suffered from various health complaints and the most common was ARI (39.2%). Health complaints experienced by the subject can hamper work productivity (Agung 2008).

Quality of life. According to Fayers and Machin (2007), quality of life is a component of happiness and satisfaction with life. The definition of quality of life is often of a different meaning for each person because it has many influencing factors such as finance, safety, and health. For this reason, a term of quality of life-related to health is used in the field of health. Assessment of quality of life is not only influenced by physical conditions, but also by mental, social and emotional states so that it can be seen as a multidimensional concept consisting of three main areas namely physical, psychological (cognitive and emotional), and social (Loonen *et al.* 2001). Assessment of quality of life provides new insights in the assessment of long-term outcomes

Table 2. Distribution of subjects based on nutritional status and health status

Nutritional status	n	%
Body Mass Index (kg/m^2)		
Thin (<18.5)	3	2.6
Normal (18.5–24.9)	51	44.0
More (25–26.9)	20	17.2
Obesity (≥27)	42	36.2
Average±SD	25.8±3.8	
Non anemia (Hb>12 g/dl)	83	71.5
Anemia		
Mild (Hb 11.0–11.9 g/dl)	24	20.7
Moderate (Hb 8.0–10.9 g/dl)	9	7.8
Average Hb±SD (g/dl)	12.6±1.1	
Health status	**n**	**%**
Infectious Disease		
Tuberculosis	3	2.5
ARI* (cough, runny nose)	95	81.9
Helminthiasis	6	5.2
Non-communicable diseases		
Joint pain	85	73.3
Hypertension	46	40.0
Uric acid	46	40.0
History of health conditions for the past four weeks		
Pain free	19	16.4
Sickness	97	83.6
ARI (cough, cold)	38	39.2
Fever	3	3.1
Hypertension	3	3.1
Stomach pain	13	13.4
Joint pain	12	12.4
Headache	14	14.4
Etc	14	14.4

ARI: Acute Respiratory Infections

based on the definition of health, according to the World Health Organization (WHO), which is physically, mentally, and socially healthy, not only free from disease or weakness. Assessment of the quality of life of the subjects in this study was carried out using a standardized Short Form 36 (SF-36) questionnaire. Quality of life in this study consists of eight dimensions, namely physical function, physical role, emotional role, vitality, mental health, social function, pain, and general health. Also, quality of life is divided into two major components, namely the Physical Component Summary (PCS) and the Mental Component Summary (MCS). Table 3 shows that the average score of the highest quality of life of subjects was in the dimensions of physical function (88.8±17.2), and the lowest was in the dimension of pain (62.1±22.9). Meanwhile, the average PCS subject score was 70.4±18.0, and MCS was 78.0±15.2. Table 3 also shows that more than half of the subjects had a relatively good quality of life (62.1%), and only 37.9% of the subjects classified as having a poor quality of life.

Table 3. The average score of quality of life of the subjects by dimensions and the distribution of subjects by the quality of life categories

Dimension	Average	Elementary school
Physical function	88.8	17.2
Physical role	66.4	44.1
The role of emotion	81.9	36.4
Vitality / energy	69. 5	13.5
Mental health	74.7	14.5
Social function	86.0	18.7
Pain	62.1	22.9
General health	64.2	10.9
PCS	70.4	18.0
MCS	78.0	15.2
Quality of life category	n	%
Good (scores ≥74)	72	62.1
Poor (score <74)	44	37.9
Average±SD	74.2±14.7	

PCS: Physical Component Summary; MCS: Mental Component Summary

Socioeconomic characteristics and the quality of life. Table 4 shows that there was no relationship between age, education level, marital status, and subject expenditure with physical components (PCS) and mental components of quality of life (MCS) (p>0.05). This result is in line with the study of Al-Aboudi *et al.* (2015), which shows that there was no significant relationship between age, education level, and health-related quality of life. However, the results of Bjorner's research (2013) on 3,445 adult subjects in Massachusetts, Illinois, and California showed a different thing that there was a strong relationship between high quality of life scores with younger subject's age, marital status, and education level. Table 4 also shows that there was a relationship between the number of family members and the PCS score (p=0.003, r=0.277). The greater the number of family members, the better the quality of life score of the subject based on the dimensions of physical role and PCS. This means that the greater number of family members does not reduce the amount of time and limit the subject to work/activities related to the physical.

Nutritional status and quality of life

The eight dimensions of quality of life related to health can be concluded into two parts,

Table 4. Relationship of subject's socioeconomic characteristics with quality of life dimensions

Characteristics	Correlation coefficient	Quality of life	
		PCS[3)]	MCS[4)]
Age	r	0.084	-0.011
	p[1)]	0.372	0903
A long time of school	r	-0.049	-0.119
	p[1)]	0601	0.203
Marital status	r	-0.122	-0,077
	p[2)]	0.193	0.413
Spending	r	0.135	0.11
	p[1)]	0.149	0.238
Number of family members	r	0.277	-0.018
	p[1)]	0.003*	0.847

[1)]Pearson correlation test; [2)]Rank-spearman correlation test; *significantly related to p<0.05; [3)]PCS: Physical Component Summary; [4)]MCS: Mental Component Summary

namely Physical Component Summary (PCS) and Mental Component Summary (MCS). PCS consists of dimensions of physical function, physical role, pain, and general health. MCS consists of vitality, social functions, the role of emotions, and mental health. The nutritional status of the subjects was assessed based on body mass index and hemoglobin levels. Table 5 shows that the average PCS scores in the nutritional status of thin, normal, overweight, and obesity were 71.9±21.3, 68.5±19.6, 75.0±12.7, 70.6±18.1, respectively. The highest average PCS scores were in subjects with overweight nutritional status, and the lowest was in subjects with normal BMI. These results are in line with the study of Ul-Haq *et al.* (2012), which showed that quality of life scores improved in overweight subjects, but decreased in obese subjects. Research Brown *et al.* (2000) in Australia show different things. Subjects who had a normal BMI (18.5–25 kg/m^2) has the highest scoring average for the domains of physical functioning, general health, and vitality on the SF-36 questionnaire. According to Brown *et al.* (2000), women of childbearing age who have excess BMI tend to have more health complaints such as hypertension, asthma, headaches, back pain that can affect the subject's decreased perception of the quality of life. This difference in results is thought to be caused because, in this study, half of the subjects who had a normal BMI had anemia (45.5%) and were ill in the last four weeks (42.3%).

Table 5 shows that the mean MCS scores on thin, normal, excessive, and obese nutritional status were 67.2±11.4, 79.4±14.4, 77.2±14.8, 77.5±16.5. The highest average MCS score is in normal nutritional status, and the lowest is in subjects with underweight nutritional status. These results are in line with the research of Wee *et al.* (2010) on Asian ethnic subjects (Chinese, Malay, and Indian) in Singapore, which showed that subjects with lean BMI were associated with MCS scores that were lower by 1.3 points compared to other BMI. Research Huang *et al.* (2006) in Taiwan showed that subjects with both overweight and obese BMI had higher mental component scores compared to those with normal BMI. The increase in BMI is followed by increasing MCS scores can be explained by socio-cultural factors such as values, attitudes, beliefs, and people's perceptions of body weight. Eastern culture views being overweight as something normal, healthy, beautiful, and a symbol of prosperity and happiness. This research also shows subjects tend to only begin to realize the dangerous health consequences when the subjects are hugely overweight.

The Pearson correlation test showed that there was no relationship between body mass index (BMI) and the dimensions of quality of life ($p>0.05$) (Table 6). This result is in line with the research of De Zwaan *et al.* (2009), which states that there is no relationship between BMI with quality of life-related to health. However, Ul-Haq *et al.* (2013) showed different results; namely, there was a relationship between BMI and quality of life-related to health where the subjects with higher BMI tend to have lower PCS and MCS scores.

Table 6 also shows that there is no relationship between anemia status and quality of life ($p>0.05$) this presumably because hemoglobin

Table 5. Average nutritional status scores based on the quality of life

Nutritional status	Quality of life	
	PCS*	MCS*
Thin	71.9±21.3	67.2±11.4
Normal	68.5±19.6	79.4±14.4
Overweight	75.0±12.7	77.2±14.8
Obesity	70.6±18.1	77.5±16.5

*PCS: Physical Component Summary; *MCS: Mental Component Summary

Table 6. The relationship of nutritional status with quality of life

Nutritional status	Correlation coefficient	Quality of life	
		PCS*	MCS*
Body Mass Index (BMI)	r	0.032	-0.007
	p[1)]	0.734	0.94
Anemia Status (Hb)	r	0.002	0.065
	p[1)]	0.986	0.49

[1)]Pearson correlation test; *PCS: Physical Component Summary; *MCS: Mental Component Summary

levels in anemia subjects are classified as mild to moderate (8.0–11.9 g/dl). According to Beck *et al.* (2012), often, sufferers of mild to moderate anemia do not realize that they have anemia, so the subject tends to work as usual and does not interfere with the daily activities of the subject. Moreover, research by Locatelli and Del Vecchio (2014) showed that worsening scores on each dimension of quality of life in subjects with anemia was significant when their Hb was <9.0 g/dl. Thus, subjects with mild to moderate anemia tend not to have significant clinical effects because the subjects have reached a more stable state. A more stable hemoglobin level will affect the subject's better perception of his/her quality of life.

Health status and quality of life

Table 7 shows that there was a significant relationship between health conditions in the past four weeks with PCS scores (r=0.39; p=0.001) and MCS (r=0.229; p=0.014). Most subjects in the last four weeks experienced health problems with symptoms including fever, cough, sore throat, coryza (runny nose), and shortness of breath. This might have been caused by the weather during the data collection, which was in the rainy season. In addition, the condition of most of the sick subjects also affects the MCS scores. This is possible because when suffering from Acute Respiratory Infections (ARI), subjects tend to limit social activities such as visiting relatives, neighbors, studying, and social gatherings to prevent disease transmission thus increases the feeling of isolation.

Table 7. Relationship of health status with quality of life

Health status	Correlation coefficient	Quality of life	
		PCS[1)]	MCS[2)]
Acute Respiratory Infection (ARI)	r	-0.118	-0.059
	p	0.209	0.527
Joint pain	r	-0.054	-0.121
	p	0.563	0.197
Health conditions for the past 4 weeks	r	0.309	0.229
	p	0.001*	0.014*

*The Spearman Rank correlation test is significantly related to p<0.05

[1)]PCS: Physical Component Summary; [2)]MCS: Mental Component Summary

The results of this study are in line with the study of Linder and Singer (2003) which showed that subjects who experience symptoms of fever, cough, sore throat, coryza (colds), and shortness of breath tend to experience a significant decrease in quality of life scores (p<0.001). The decrease in quality of life scores in this study not only on the PCS scores (Dimension of physical function, physical role, body pain, and vitality), but there is also on the MCS scores (Dimension of social function, and mental health) (Linder & Singer 2003).

Factors affecting the quality of life

The results of the multiple logistic regression test showed that the variables that affect the subject's quality of life were the number of family members and subjects' expenditures (Table 8). Subjects who have a large number of family members (≥4 people) are had 3.5 times higher risk to experience lower quality of life compared to subjects who have small families (<4 people) (OR=3.52; 95% CI:1.23–10.05). The small number of family member enables family to increase social status. Families increasingly have the chance to send their children to higher education, increased health status, and more excellent savings opportunities so that it will have an impact on the quality of life that is increasing. Research by Santos *et al.* (2016) also showed that workers who lived with more than four people were associated with a lower quality of life. A large number of family members allow a person to be overloaded with work, which can affect health.

Table 8 shows that subjects with monthly expense of >Rp. 343,646 had lower risk of experiencing low quality of life 59.7% compared to subjects who had household an expense of

Table 8. Logistic regression analysis of subjects' quality of life

Variable	p	OR	95% CI
Number of family members	0.019	3,521	1,233–10,052
Total expenditure	0.041	0.403	0.168–0.964

<Rp. 343,646 (OR=0.403; 95% CI:0.17–0.96). The result implies that higher income has led to lower risk of deteriorating quality of life. However, the size of income alone is not enough to determine health status and quality of life of the household. According to Sulistyowati *et al.* (2017), the composition of household expenditure can be used as a measure to assess the level of the economic welfare of the community, the higher the percentage of expenditure for food against total expenditure, the lower the welfare of the community. This study found the proportion of the household expenditures were as follow (1) basic food (15.8%), (2) cigarettes (12.7%), (3) education (9.7%), (4) snacks (11.9%), (5) installments/credit (8.5%), and (6) health (3.2%).

We found the expenditure for cigarettes in our survey was almost the same to the proportion of income allocate for food. According to Semba (2006), poor household heads who smoke reached 73.8%, and spending on tobacco reach 22% per capita per week of total the household expenditure. Increased household expenditure to buy cigarettes has strained the ability of the households to meet their basic needs, such as food consumption, education, and health. This lead to deteriorating health conditions, causes anxiety and discomfort; furthermore, this can affect a person's quality of life (Do & Bautista 2015).

CONCLUSION

Most subjects classified as having a body mass index (BMI) >25 kg/m^2 (obese). The prevalence of anemia in this study was 28.5%, with an average hemoglobin level of 12.6 g/dl. The most common infectious and non-communicable diseases suffered by the subject are Acute Respiratory Infection (ARI) and joint pain. Most subjects suffered pain in the past four weeks. Most subjects had a relatively good quality of life, with an average score of 76.3±13.3. There is a positive relationship between the number of family members with physical role dimensions and Physical Component Summary (PCS). There is no significant relationship between nutritional status and anemia status with quality of life. There is a positive relationship between ARI with dimensions of social function and joint pain with the role of emotions. There is a positive relationship between the health conditions of the past four weeks with dimensions of physical roles, general health, vitality/energy, mental health, PCS, and MCS. Factors that influence the quality of life of the subject are the family size and total household expenditure.

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AUTHOR DISCLOSURES

The authors have no conflict of interest.

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Breastfeeding Practices among Mothers at Birth and at 6 Months in Urban Areas of Delhi-Ncr, India

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ABSTRACT

The objective of the present was to potray the breastfeeding practices followed by breastfeeding mothers, at birth and at 6 months, in urban areas of Delhi-National Capital Region. For this, 185 dyads of mother-infant aged 6 months were selected from Pediatric Outpatient Department of a government and a private hospital. A questionnaire-cum-interview was designed to collect the participants' information on breastfeeding practices. It was found that only 29.7% infants had early initiation of breastfeeding despite the high (78.9%) institutional deliveries. Around 40.5% infants were given feeds apart from breast milk, mainly infant formula (81.3%) at the hospital after birth. There were 13% infants who received pre-lacteal feeds, mainly as a traditional family custom. The practice of exclusive breastfeeding for first six months was followed by 62.2% mothers. Complementary feeding or top feeding was received by 71.9% infants, but only 45.1% mothers initiated complementary foods at the recommended age of 6 months. It can be concluded that in the urban areas of India breastfeeding practices remains poor even after rigorous work done for promotion and protection of breastfeeding. Hence strong institutional care and support for breastfeeding is required at birth and efficient counseling for appropriate feeding of the infant is needed to intensify breastfeeding.

Keywords: breastfeeding, breast milk, complementary feeding

INTRODUCTION

Breastfeeding practices like initiation of breastfeeding within one hour of birth and exclusive breastfeeding for the first six months of life have immense public health importance (Gupta *et al.* 2019). Breastfeeding has nutritional, immunological, behavioral and economic benefits and also provide desirable mother infant bonding (Shili *et al.* 2012). Breastfeeding offers adequate nutrition for the baby as well as build up the bond between mother and their baby (Pangestuti 2018). The beneficial effects of breastfeeding depend on time of breastfeeding initiation, its duration and the age at which the breastfed child is weaned (Victoria *et al.* 1987). The Lancet 2013 report showed that optimal breastfeeding has a greater role in averting 13% of all deaths under the age of 5 years in developing countries (UNICEF 2015). Optimal breastfeeding practices are potentially one of the top interventions for reducing under-five mortality and is essential for the achievement of many of the newly launched Sustainable Development Goals by 2030, as it can help to improve child and maternal health, nutrition, economy, intelligence, and human capital, while reducing inequalities (Sultania *et al.* 2019).

Colostrum, the milk secreted in the first 2–3 days after delivery, acts as the first immunization right after birth for the newborn. Colostrum is rich in white cells and antibodies, especially sIgA, and it contains a larger percentage of protein, minerals and fat-soluble vitamins (A, E, and K) than later milk (Polineni *et al.* 2016). Hence early initiation of breast feeding is essential for the infant to avail of the benefit of colostrum.

Exclusive Breastfeeding (EBF) is defined as giving no food to the infant except breast milk except oral rehydration solution, vitamins, minerals or medicines (WHO 2003). EBF for the first six months of life is the cornerstone of optimum infant nutrition (WHO 2008). It reduces the risk of the infant to experience diarrheal diseases (Ogbo *et al.* 2017; Ogbo *et al.* 2017; Victoria *et al.* 2016; Ogbo *et al.* 2018), upper respiratory tract infections, obesity in later life, and EBF could also improve the neurocognitive functions of the child (Victoria *et al.* 2016). Breastfed babies have less chance of allergies, asthma and eczema. Evidence suggests that exclusive breastfeeding for at least two months

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protects susceptible children from Type I Insulin Dependent Diabetes Mellitus (IDDM) (Polineni *et al.* 2016).

In ancient time, breastfeeding was the only source of feeding of newborn, but during industrialization, urbanization and modernization, this practice has gradually declined (MoHFW, GOI 2013). Studies have shown that EBF rates were influenced by mother's education, age, and employment; infant's age, sex; access to healthcare; neighborhood of residence; and exposure to mass media or counselling (Martines *et al.* 1989; Kimani-Murage *et al.* 2011; Tamiru *et al.* 2013, Setegn *et al.* 2012; Mekuria & Edris 2015).

Though there has been an improvement in the breastfeeding status in India over decades due to strengthening of policies, effective capacity building initiatives, community-based actions and strategic mass media communication (Aguayo *et al.* 2016), further progress is necessary as highlighted in National Health and Family Survey (NFHS)-4 (2015-16). Data shows that only 41.6 percent children are breastfed within first hour of birth in spite of a substantial increase in the rates of institutional deliveries from 38.7 per cent (NFHS-3) to 78.9 per cent (NFHS- 4) during a span of ten years. Also, nearly half (45.1%) the children under six months of age are not exclusively breastfed for the first six months (IIPS 2017). NFHS-4 also shows that 21% newborns receive pre-lacteal feeds and about 22% babies are born with low birth weight, who need extra support. Many of the current institutional birth in India were done in private facilities. While, evidence suggest that introduction of infant formula in private health facilities is a common practice (Gupta & Thankur 2018).

Thus, to achieve optimum breastfeeding status, it becomes imperative to first understand the breastfeeding practices followed by mothers and the healthcare providers. So, the objective of the present study is to investigate the breastfeeding practices followed by breastfeeding mothers when their babies were at birth and at 6 months in Urban Delhi-NCR.

METHODS

Design, location, and time

The present study was a descriptive cross-sectional study undertaken at the Outpatient Pediatric Department (OPD) of a private clinic and Government Hospital in Gurugram (NCR). The data was collected from March 2017 to December 2017.

Sampling

A total of 185 mother-infant (6 months) dyads attending Pediatric OPD of two sites i.e. 100 from a private clinic, Gurugram and 85 from public hospital, of Gurugram were selected purposively due to their high OPD rate to assess the breastfeeding practices followed by mothers when their babies were at birth and at 6 months in Delhi-NCR. Infants aged less than or more than 6±0.5 months, infants suffering from any severe chronic disease or disorder, twins, low birth-weight, pre-term and, infants never breastfed were not included in the study.

Data collection

The tool for data collection used was a detailed questionnaire followed by structured interview designed to record the respondents' socio-demographic profile, infants' details, maternal details, information on breastfeeding practices i.e. early initiation of breastfeeding, top feeds and pre-lacteals given, exclusive breastfeeding for six months, continued breastfeeding, bottle feeding and initiation of complementary feeding. Retrospective data was collected to get information about early breastfeeding practices of mothers around birth.

Participant information sheet was given to the enrolled respondents and a written informed consent was obtained before conducting the interview. The participant information sheet was read out to mothers who were illiterate, and their thumb prints were taken on informed consent sheet. Written permission was also obtained from both of the study sites i.e. private clinic, Gurugram and District Civil Hospital, Gurugram for conducting the study. Ethical clearance (IHE/2017/Ethics/019) was granted from the Institutional Ethics Committee of the Institute of Home Economics, University of Delhi.

Data analysis

Data coding and data entry was done using in MS Excel. Frequency and percentages were calculated for the participants' socio-demographic profile, infants' details, maternal details, immunization details, IYCF practices.

Mean and standard deviations were calculated for continuous variables.

RESULTS & DISCUSSION

Socio-demographic details

Infants. The results of the present study highlighted that of the total 185 infants, 63.7% were males and 36.2% were females respectively, with majority (92.4%) being Hindus. The mean (SD) age of the infants was 6±0.52 months and the mean (SD) birth weight was 2.8±0.54 kg. No significant difference (p=0.13) was found between the birth weight of infants from Civil Hospital and private hospital clinic. Most (53%) of the infants were delivered at Government Hospital followed by 44.3% at Private hospital. Regarding the mode of delivery, 62.7% were vaginal deliveries while 37.2% were C-section deliveries. Nearly half (46.5%) of the infants were the first-born child (Table 1).

Mothers. Maternal age ranged from 17 to 38 years and nearly half (55.4%) the mothers were between the age of 23 to 27 years. Twenty-six per cent mothers were graduates followed by 22.7% who had senior secondary level of education. Only 12.4% were illiterate. Majority (91.4%) of the mothers were housewives. There were 48.1% mothers who had only one child and 36.8% with two children. Amongst all mothers, 56.2% belonged to joint families but more mothers i.e. 61.2% attending civil hospital had a nuclear family as compared to 29% mothers attending private hospital. This could be because mothers attending Government Hospital originally belonged to other states and settled in Delhi-NCR with their husband and children. More than half (55.1%) of the participants had four to six members in the family (Table 2).

Table 1. Characteristics of infants 6 months of age visiting pediatric Outpatient Pediatric Department (OPD) of private clinic and civil hospital, Gurugram

Characteristic	Frequency (n=185)	Percentage
Gender		
Males	118	63.7
Females	67	36.2
Religion		
Hindu	171	92.4
Muslim	13	7
Sikh	1	0.5
Place of delivery		
At home	5	2.7
Private hospital	82	44.3
Government hospital	98	53
Type of delivery		
Vaginal	116	62.7
C-section	69	37.2
Birth Order		
First	89	48.1
Second	68	36.8
Third	22	12
Fourth	6	3.2

Early breastfeeding practices

Early initiation of breastfeeding was found to be sub-optimum in the present study (Figure 1). Around one-third (29.7%) of the infants were fed breastmilk within one hour of the birth. Among the mothers attending pediatric OPD of private clinic, only 22% reported to feed their infant with breastmilk within first one hour of birth while 38.8% mothers from civil hospital did so. In the case of C-section deliveries, 87% of the infants first received breastmilk after two or more days. Regarding colostrum feeding, 94.1% of infants received it after birth with 92.4% from private hospital and 91% from civil hospital. Two-third (69.7%) of the mothers fed it because it was suggested by the doctor/health worker followed by only 28% reported feeding it as it is good for the immunity of the baby. Half the mothers who did not feed colostrum to their babies followed the advice of the doctors/health workers, who suggested them not to give their colostrum to their baby. Only 13% infants received pre-lacteals such as honey, ghutti and it is mostly as a traditional practice followed in the family (75%). About 45% of the infants were given feeds other than breastmilk, infant formula being the most commonly (81.3%) given at the hospital right after birth. When analyzed according to the place

Table 2. Socio-demographic characteristics of mothers visiting pediatric Outpatient Pediatric Department (OPD) of private clinic and civil hospital, Gurugram

Characteristic	Frequency (n=185)	Percentage
Age (years)		
17−22	36	19.5
23−27	97	52.4
28−32	41	22.2
33−37	11	6
Education		
lliterate	23	12.4
Primary school	7	3.8
Middle school	21	11.4
Secondary school	27	14.6
Senior secondary school	42	22.7
Graduation	48	26
Post-graduation	27	14.6
Livelihood		
Working	16	8.6
Non-working	169	91.4
Parity		
1	89	48.1
2	68	36.8
>2	28	15.1
Type of family		
Nuclear	81	43.8
Joint	104	56.2
Size of family		
<4	47	25.4
4−6	102	55.1
>6	36	19.5

of birth, 36.5% infants from Government hospital and 53% infants delivered in private hospital received top feeds such as infant formula, animal milk, pulse water.

Breastfeding practices at 6 months

Majority of the mothers (92.4%) were breastfeeding their child at the age of 6 months and only 7.5% mothers discontinued it by 5 months of age. Insufficiency of breastmilk was the primary reason for discontinuing breastfeeding as reported by 71.4% mothers from both private clinic and Government hospital who no longer breastfeeding. There were 46.2% mothers who were feeding breastmilk to their child for more than 12 times a day. When asked about the sufficiency of breastmilk for fulfilling all nutritional requirements of the infant at 6 months, 68.6% mothers suggested that breastmilk alone is not enough for the proper growth and development of the baby and hence foods other than breastmilk needs to be given to the child (Table 3).

Exclusive breastfeeding and complementary feeding

Table 4 highlights the exclusive breastfeeding and complementary feeding status of the mothers. Overall 62.2% infants were exclusively breastfed for the first 6 months or beyond. Almost similar percentage of mothers i.e. 61% from both private clinic and 63.5% from civil hospital reported exclusively breastfeeding their child. Complementary feeding/top feeding was initiated by 71.8% mothers mostly because they felt that the baby was hungry even after breastfeeding. But only 32.4% mothers initiated complementary feeding at an appropriate age of 6 months. Bottle feeding was reported among 23.8% infants. When inquired about the foods

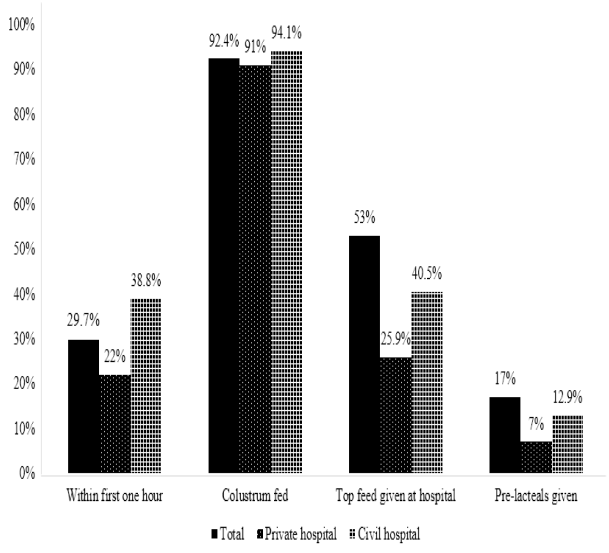

Figure 1. Early breastfeeding practices amongst mothers of infants 6 months of age

Table 3. Breastfeeding practices amongst mothers of infants at 6 months

Breastfeeding Practices (BF) at 6 months	Frequency (n=185)	Percentage
Continued BF at 6 months	171	92.4
Frequency of BF/day	(n=171)	(n=171)
<3 times	2	1.2
3−6 times	7	4.1
6−9 times	30	17.5
9−12 times	53	31
>12 times	79	46.2
Duration of BF before discontinuation	(n=14)	(n=14)
<3 months	4	28.6
3−5 months	10	71.4
*Reasons to stop BF	(n=14)	(n=14)
Age of the infant	1	7.1
Child stopped himself/herself	4	28.6
Insufficient breast milk	10	71.4
Mother was sick	1	7.1
Mother resumed work	1	7.1
Breast milk sufficient at 6 months (mother's perception)		
Yes	58	31.3
No	127	68.6

*Multiple responses

given to the infant in the past 24 hours, 35.7% infants received breastmilk only, 34.6% infants received semi-solid foods and 27.6% infants were given animal-source milk. These foods were mainly given as the mothers felt that the baby was hungry or thirsty, advised by doctor/ health worker or by mother/mother-in-law.

Appropriate feeding is crucial for the healthy growth and development of the infants. Breastmilk is the natural first food for babies. It continues to provide upto half or more of the child's nutritional needs during the second half of the first year, and upto one third during the second year of life (WHO 2009). In the present study few early breastfeeding practices were found to be sub-optimal even after 97.3% institutional births. Provision of mother's breast milk to infants within an hour of birth is referred to as "Early Initiation of Breastfeeding" (EIBF). This ensures that the colostrum or "the first milk," which is rich in protective factors, is given to the infants immediately after delivery. In developing countries like India, EIBF could save about 1.45 million lives a year, attributed to various infections (Lauer *et al.* 2006). Early initiation of breastfeeding was as low as 22% in the present study. Shili and co-authors conducted a study in rural areas of Uttarakhand to know breast feeding practices of mothers and to strengthen these practices among 500 mother infant dyads. They also found that only 21.37% of the infants received breastmilk within first one hour of birth (Shili *et al.* 2012). Major barriers to early initiation of breast feeding includes lack of awareness regarding proper technique of breastfeeding and benefits of colostrum, breast abnormality like inverted/retracted nipples, obstetric/neonatal complications requiring

Table 4. Exclusive breastfeeding and complementary feeding practices amongst mothers of infants at 6 months

Indicators	Frequency (n=185)	Percentage
Exclusive breastfeeding untill 6 months or beyond	115	62.2
Children receiving complementary feeding (CF)	133	71.8
Initiation of CF at 6 months	60	32.4
Bottle feeding	44	23.8

specialized care, and cultural practices like giving pre-lacteals and gender discrimination (Majral & Silan 2016). Breast feeding initiation can be particularly delayed for infants born by caesarean section. Prospective cohort studies in India have shown that infants born by caesarean section were almost four times less likely to initiate breast feeding within 1 hour of birth than infants born by vaginal delivery (Patel *et al.* 2015). Only 13% infants delivered by C-section received breastmilk within first one hour of the birth. This indicates that adequate support is not provided to mother during caesarean section delivery which acts as a barrier in early initiation of breastfeeding. However, colostrum was given to majority (92.4%) of the infants which is an improvement based on the Infant and Young Child Feeding (IYCF) recommendations. Similar findings were reported by Polineni *et al.* where 96.3% mothers fed colostrum to the infants (Polineni *et al.* 2016). But 69.7% mothers gave colostrum as it was suggested by the doctor/health which reflects that there is a lack of knowledge among mothers about the benefits of colostrum feeding in the present study. Pre-lacteals such as honey, ghutti was given to only 13% infants mainly as a traditional practice in the family which can be considered as a success in IYCF counseling in India. On contrary to this, Shili and associates reported that pre-lacteals such as sugar water, honey and gripe water were given to 61.8% infants (Shili *et al.* 2012).

While continued breastfeeding at 6 months was observed among 92.4% mothers, exclusive breastfeeding was practiced by 62.2% mothers which is more than the figure (54.9%) reported in NFHS-4 (IIPS 2017). The results are similar to another study carried out by Kumari *et al,* in Telangana where exclusive breastfeeding was followed by 60.6% mothers (Kumari *et al.* 2017). In another study in Karnataka only 40% mothers were exclusively breastfeeding their babies (Madhu *et al.* 2009). The most common reason seen for discontinuing exclusive breastfeeding before six months is insufficiency of breastmilk as perceived either by the mother herself or the family members.

In the present study 71.8% infants received complementary foods/top milk but only 32.4% received them at the appropriate age of six months. Either early or late introduction of the complementary foods was observed. This shows the lack of knowledge among the mothers regarding appropriate age of initiation of complementary feeding and to certain extent the pressure from family members as the majority of the mothers belonged to joint family. The insufficiency of breast milk was a major concern for the grandmothers who recommended their daughter/ daughter-in-law to start giving the child other foods before six months (Fjeld *et al.* 2008). Likewise Aggarwal *et al.* reported that only 17.5% mothers started complementary feeding at the recommended time (Aggarwal *et al.* 2008). On the other hand among 200 mothers, 77.5% started complementary feeding at the recommended age of six months as highlighted in another hospital based study in Mangalore (Rao *et al.* 2011)

CONCLUSION

It can be concluded that the present study focuses on the need of support provided for early initiation of breastfeeding to the mothers at the health facility especially for those who had caesarean section deliveries. The practice of giving top feeds at the hospital right after the birth should be discouraged and a more stringent monitoring system should be developed to prevent these malpractices. Though there seems to be an improvement in exclusive breastfeeding, further education and awareness should be provided during ante-natal and post–natal counselling to mothers as well as the family members regarding the benefits of early and exclusive breastfeeding and continued breastfeeding till two years of age

as well as the proper time for complementary feeding initiation.

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AUTHOR DISCLOSURES

The authors have no conflict of interest.

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In-Vitro Alpha Amylase Inhibition and Antioxidant Activities of Leaves Extract of Sundanese Traditional Salad (*Lalapan*) from Indonesia

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ABSTRACT

This research aims to evaluate the α-amylase inhibitory activity and antioxidant potential of leaves extract of Gandaria (*Bouea macrophylla* Griff.), Basil (*Ocimum africanum* Lour.), Pohpohan (*Pilea melastomoides* (Poir.) Wedd.), and Lettuce (*Lactuca sativa* L.) as common vegetables used in Sundanese traditional salad. Extraction methods used were traditional and maceration methods. Phytochemical screening was used to determine the phytochemical component qualitatively. Quantitative estimation of phenols (Folin-Ciocalteu method), flavonoids ($AlCl_3$ colorimetric assay), total antioxidant activity along with free radical scavenging activity (DPPH) was also carried out. Soluble starch was used as substrate for analysis of α-amylase inhibitory activity, which is calculated by IC_{50} value. Phytochemical screening revealed the presence of phytochemical constituents like flavonoids, tannins, saponins, and polyphenols compound in both extraction methods. The results showed that the value of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), antioxidant activity, and α-amylase inhibitory in both extraction were significantly different for each leaves extracts ($p<0.05$). The gandaria leaves extracted with maceration method had the highest value among the leaves analysed. The value of TPC, TFC, antioxidant activity were 364.56±65.97 mg Gallic Acid Equivalent (GAE)/g extract, 70.2±10.54 mg Quercetin (QE)/g extract, and 35 µg/ml of IC_{50}, respectively. The extract of gandaria maceration leaves exhibited significant α-amylase inhibitory activity with an IC_{50} value of 60 µg/ml. Various pharmacologically active compounds were found in the local vegetables, thus they can be rich sources of antioxidants and α-amylase inhibitor activity. Hence, could be developed as vegetables based functional food products.

Keywords: α-amylase, antioxidant, extraction methods, *lalapan*

INTRODUCTION

Diabetes mellitus is one of the diseases associated with an increased production of Reactive Oxygen Species (ROS) and impaired antioxidant defense system which result in oxidative damage (Kundusen *et al*. 2011). Diabetes mellitus is also characterized by hyperglycemia with disturbances of carbohydrate, lipid, and protein metabolism resulting from defect in insulin secretion and/ or insulin action (Keerthana *et al*. 2013). Thus reducing the postprandial hyperglycemia to lower glucose absorption using inhibitors of carbohydrate digesting enzymes is important (Bhuvaneshwari *et al*. 2014). The α-amylase is an enzyme to break down starch into simple sugars, such as dextrin, maltotriosa, maltose, and glucose. Inhibiting the activity of α-amylase enzyme is an effective method to control blood glucose (Alexander & Maltodextrins 1992). Bioactive components found in herbal plants have shown good antioxidant and anti-diabetic properties (Kunyanga *et al*. 2012). As a bioactive compound, phytochemicals components such as tannins, phenols, and flavonoids have potential as inhibitors of the α-amylase enzyme (Keerthana *et al.* 2013).

Blood glucose can be controlled by maintaining diet or dietary behavior, regular physical activity, maintaining body weight, and consuming vegetables that can help regulate blood glucose concentration effectively (Prabhakar & Doble 2008). *Lalapan* (Sundanese traditional salad) is a raw and fresh vegetable dish commonly consumed by Sundanese in their daily meal. Pratama (2014), identified that the most common vegetables served as part of this traditional salad

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were basil, pohpohan, and lettuce leaves. Another leave commonly consumed by Sundanese and is containing phytochemical compounds and health benefits is gandaria leaves (Lolaen *et al*. 2013; Rajan & Bhat 2016). Previous research on antioxidant activity showed that the leaves of gandaria, basil, pohpohan, and lettuce have a high antioxidant activity (Andarwulan *et al.* 2010; Marwat *et al*. 2011; Gan & Azrina 2016; Andina & Musfirah 2017).

Considering the local availability and eating habit as well as findings on the potential health benefits of these leaves, it is important to explore the bioactive components potential for health. The result can inform the development of functional food based on locally available vegetables. The exploration of the leaves bioactive components should also consider the extraction method since the extraction method will affect the extracted compound. There are two extraction methods according to Verawati *et al*. (2016), they are the traditional and the laboratory or maceration methods. The traditional and the maceration methods are often used because they are relatively easy to conduct and do not require special tools. However, studies have shown that extraction processes using either the traditional or the maceration methods affected the total phenolic compound extracted from the leaves (Verawati *et al.* 2016). Therefore, it is imperative to identify the antioxidants and α-amylase inhibitory activity of the Sundanese traditional salad (*Lalapan*) using both the traditional and the maceration extraction method.

METHODS

Design, location, and time

This experimental study used a randomized block design. The research activities included sample extraction, phytochemical screening, analysis of phenol and flavonoid, antioxidant activity, and inhibition of α-amylase carried out in the Laboratory of Chemistry and Food Analysis, Department of Community Nutrition, IPB University, started from October–January 2019.

Materials and tools

The gandaria leaves was collected from Indonesia Institut of Science, Cibinong Bogor, while basil, pohpohan, and lettuce leaves were collected from Cigombong Agricultural Technology Park, Bogor. Specimens have been authenticated (No:2115a/IPH.1.01/If.07/XII/2018) with the following results are Gandaria (*Bouea macrophylla* Griff.), Basil (*Ocimum africanum* Lour.), Pohpohan (*Pilea melastomoides* (Poir.) Wedd.), and Lettuce (*Lactuca sativa* L.).

Materials used for extraction and analysis were water for solvent, aquades, container, Wagner reagents, Liebermann-Burchard reagent, HCl, methanol, Mg, KOH, $FeCl_3$, gallic acid, Folin-Ciocalteu, Na_2CO_3, quercetin, $AlCl_3$, $NaNO_2$, NaOH, vitamin C, 1.1-diphenyl-2-picrylhydrazyl (DPPH), porcine pancreatic α-amylase enzyme concentration of 30 U/mg, 1% starch, phosphate buffer pH 6.9, 3.5-dinitrosalicylic solution (DNS), and potassium sodium tartrate.

The tools used for extraction and analysis were analytical scale, knive, board, filter cloth, Whatman 41, Buchner, blender, centrifuge, hot plate, and vacuum evaporator, erlenmeyer, test tubes, micro pipet, cuvettes, vortice, water bath, and UV-VIS spectrophotometers (Shimadzu UV-160A, Kyoto, Japan).

Data collection

Sample preparation and extraction of leaves material. Sorted leaves were dried in shade at room temperature to choose the edible parts, leaves were washed in tap water and drained at room temperature, then leaves were chopped into small pieces to obtain a larger surface area. The extraction method used were traditional extraction and the maceration (laboratory). In the traditional extraction, samples were blended with water (1:10 w/v), then centrifuged at 2,000 rpm (5 minutes) and the extract was filtered through a Whatman 41 using Buchner, and evaporated using a hot plate to obtain the liquid extract (Aisyah *et al*. 2015 modification). While in the maceration method, the sample was placed in a container and soaked in water. The container with its contents was sealed and kept for 2x24 hours with some stirring. Subsequently, the entire mixture then filtered by a filter cloth, then filtered again through Whatman 41 using Buchner. After filtration process, the macerate was evaporated at 60^{o}C (65 kPa) using vacuum evaporator to obtain the dry extract (Verawati *et al*. 2016 modification).

The evaporation process of traditional extracts aimed to make it easier to control the

concentration of the solution. Stock solution was done by weighing both extracts obtained from the two different method in the same amount. Thus, it was expected that the Total Dissolve solid (TDS) in the stock solution could be controlled despite the amount was not exactly the same due to the different sample form.

Phytochemical screening. The extracts were analyzed for the active phyto-constituents such as alkaloids, flavonoids, tannin, saponin, polyphenols, and terpenoid according to Harborne (1987). Alkaloids were analyzed by dissolving 2 ml of sample in 2 ml of 2% HCl, then heated for 5 minutes and filtered. The filtrate was added by 2–3 drops of Wagner reagent. The formation of white deposits shows the presence of alkaloid compounds. Flavonoids were analyzed by dissolving 2 ml of sample in 2 ml of methanol, then added with Mg and 5 drops of 37% HCl. The formation of red or orange colour indicates the presence of flavonoids. Tanin was analyzed by adding 2 ml of sample with $FeCl_3$ reagent. The formation of dark blue or blackish green colour indicates the presence of tannin. Saponin was analyzed by dissolving 2 ml of sampel in a test tube, 10 drops of KOH were added and then heated in a water bath (50°C) for 5 minutes and the solution was shaken for 15 minutes. The presence of a saponin is arranged with a steady foam and stable for 15 minutes. Polyphenols were analyzed by dissolving 2 ml of sample in 10 ml distilled water and heated for 5 minutes, then filtered. The filtrate was added with 4–5 drops of $FeCl_3$ 5%. The formation of dark blue or blackish green colour indicates the presence of a phenols. Terpenoid was analyzed by adding 2 ml of sample with 1 ml of the Liberman-Burchard reagent. The formation of dark blue or blackish green colour indicates the presence of terpenoid compounds.

Estimation of total phenols. One ml of the extract solution was mixed with 1 ml of Folin-Ciocalteu reagent, then 1 ml of aqueous 10% Na_2CO_3 solution was added and vortexed. The mixture were allowed to stand for 90 minutes at room temperature. Absorbance was measured using a spectrophotometer at 765 nm. The TPC was subsequently calculated using gallic acid as standard. Results obtained were expressed as mg GAE/g extract (Singleton & Rossi 1965).

Estimation of total flavonoid. One ml of the extract solution dissolved in aquades (1:4), added 60 µl of 5% $NaNO_2$ solution (incubated for 5 minutes), then added with 60 µl of 10% $AlCl_3$ solution (incubated for 6 minutes). After that, added with 400 µl of 10% NaOH solution and then prepared by aquades to 2 ml. The mixture then was vortexed. The absorbance was measured using a spectrophotometer at 510 nm. The TFC was calculated using quercetin as standard, and results obtained were expressed in term of QE/g extract (Sahu & Saxena 2013).

DPPH free radical-scavenging assay. Antioxidant activity were determined based on percentage inhibition of DPPH radical and used vitamin C as a standard. The results obtained are presented by the IC_{50} value (concentration of the sample required to inhibit 50% of radical). The lower of IC_{50} value indicates that antioxidant activity is getting higher (Molyneux 2004). 750 µl of extracts solution at different concentrations (0.25; 0.5; 1; 2; 4 mg/ml) were added to 750 µl of 0.2 mM DPPH methanolic solution, the mixture was vortexed and left to stand in the dark for 30 minutes. Absorbance was measured at 517 nm. The percentage of inhibition of DPPH radical was calculated according to the following equation:

$$\%Inhibition = \left(\frac{Abs\ control - Abs\ sample}{Abs\ control}\right) X100$$

whereas, Abs control is the absorbance of DPPH solution without leave extracts.

In-vitro α-amylase inhibitory assay. The analysis of α-amylase inhibitory was conducted using the method described by Apostolidis *et al*. (2007) with slight modification. Acarbose as the pharmacological inhibitor was included as the positive control. Thirteen µl of extracts solution at different concentrations (0.078; 0.156; 0.3125; 0.625; 1.25; 2.5; 5; 10 mg/ml) were added to 94 µl phosphate buffer (pH 6.9) containing α-amylase solution (30 U/mg). Then, 63 µl of 1% soluble starch and 207 µl phosphate buffer (pH 6.9) were added to each tube at time intervals. The mixture was incubated at 37°C for 10 minutes. The reaction was stopped with a 313 µl of DNS reagent. The test tube were then incubated at 100°C for 10 minutes and cooled to room temperature. The mixture was then diluted with 313 µl of 40% potassium-sodium tartrate. Absorbance was measured at 540 nm. The percentage of α-amylase inhibitory was calculated according to the following equation:

$$\%Inhibition = \left(\frac{Abs\ control - (Abs\ sample - Abs\ blank)}{Abs\ control}\right) X100$$

whereas, Abs control is the absorbance of solvent without sample, Abs blank is the absorbance of solvent without enzyme.

Data analysis

Data processing used the Microsoft Excel 2013 and Statistical Program Social Science (SPSS) version 16.0. Data analysis comprised of descriptive analysis, normality test, and different test. Different test were analyzed using Two Way ANOVA and Least Significance Differences (LSD) for differences of each samples ($p<0.05$). Results were expressed as the mean±standard deviation.

RESULT AND DISCUSSION

Phytochemical screening and quantification of secondary metabolite

Qualitative phytochemical screening was aimed to identify any chemical components with bioactive compounds in the plant extracts, which can be further utilized (Aharoni & Galili 2011). The qualitative analysis of the leaves extract confirmed the presence of flavonoids, tannins, saponins, and polyphenol compounds in both of the traditional and the maceration methods (Table 1).

The results of phytochemical screening showed different result from previous research, such as for mango (*Mangifera indica*) that is in the same family as gandaria (*Anacardiaceae*), basil (*O. basilicum)*, pohpohan (*Pilea trinervia*), and lettuce (*Lactuca sativa*) leaves. However it is important to note that the leaves species as well as the extraction method used in these research were varied (Adham 2015; Dhital 2017; Guntara *et al*. 2016; Algfri *et al.* 2019). Thus, difference in the phytochemical components found in this study might be caused by several factors such as differences in the extraction steps, the polarity of solvent, and the species of plant. In addition, differences in the secondary metabolites component found in plants can be due to soil conditions where the plants are planted, such as soil moisture, water availability, temperature, pH, land conditions, and light intensity (Vinolina 2014).

Phenolic compounds are the most widely distributed secondary metabolites and play an important role in growth regulators, have antioxidant, structural, attractant, signaling and protective functions (Babenko *et al.* 2019). The value of Total Phenolic Content (TPC) in both extraction methods were significantly different for each leaves extracts ($p<0.05$) (Figure 1). The results also showed that phenolic content also significantly different between each of leaves species, thus extraction methods and the leaves species affected the total phenolic content.

The results showed that gandaria leaves extract with maceration had the highest total phenolic content, followed by gandaria from traditional extraction, lettuce, basil, and pohpohan with maceration extraction. The leaves extracted with traditional method showed lower results of TPC compared to the maceration. This is probably that maceration extract was evaporated using a vacuum evaporator at 60^{o}C (65 kPa), so the water content evaporates more than in the traditional evaporation using a hot plate. The final result of maceration is dry extract obtained by drying the liquid extract until specific concentration according to the procedure. Drying is the process for removing solvents from the material to obtain powder/dry extract, while traditional extraction

Table 1. Phytochemical screening of leaves extract

Phytochemicals	Traditional				Maceration			
	Gandaria	Basil	Pohpohan	Lettuce	Gandaria	Basil	Pohpohan	Lettuce
Alkaloids	-	-	-	-	-	-	-	-
Flavonoids	+++	++	++	++	+++	++	++	++
Tannin	+++	++	+	+	+++	++	+	+
Saponins	+	+	++	+	+	++	+++	+
Polyphenols	+++	++	+	+	+++	++	+	+
Terpenoids	-	-	-	-	-	-	-	-

+ Present; - Absent

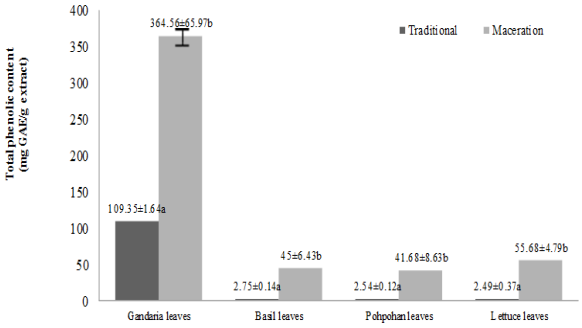

Figure 1. Total phenolic content of leave sextract. Different letters (a-b) for each leaves are significantly different ($p<0.05$)

is liquid extract so the concentration couldn't be controlled properly (Martin *et al*. 1961).

The evaporation process uses a vacuum so that it is good for food products that are sensitive to high temperatures. It can be processed to reduce their water content without damaging the physical condition and chemical content of the material (Supriatna 2008). In traditional extraction method, the extraction results were evaporated using a hot plate at 100°C without vacuum until the volume was reduced by 50% to get the liquid extract. While in the maceration, results were evaporated using an evaporator at 60°C so that the possibility of losing the water soluble nutrients will be even lower and the average of water evaporated was 98.8% by vacuum to get dry extract.

Verawati *et al*. (2016) reported that the total phenolic content of piladang water leaves extract with traditional extraction method (*S. scutellariides L.* Codd), evaporated by hot plate showed lower results (77.31±0.21 mg GAE/g extract) compared to piladang maceration leaves extract (293.30±0.13 mg GAE/g extract). Difference in extraction method affected the extracted compound. Andina and Musfirah (2017) reported that the TFC of gandaria leaves extracted by maceration was 68.53±1.37 mg GAE/g extract equal to our findings with the same method. It showed that the extraction method in different process affects the secondary metabolites content. In addition to extracted methods, differences in soil conditions or growing environment of plants will also affect the content of chemical compounds in these plants. The results of secondary metabolite content will show different on the same plant type if these plants are planted in different places (Yenni 2012).

Flavonoids are antioxidant compounds and naturally produced as secondary metabolites in plants. The Total Flavonoid Content (TFC) in different extraction methods were significantly different for each leaves extracts ($p<0.05$) (Figure 2). The results also showed that flavonoid content also significantly different between each of the leaves extracts. It means that the extraction methods and leaves species affected the total flavonoid content of gandaria, basil, pohpohan, and lettuce leaves.

The maceration leaves extract contained higher TFC compared to the traditional leaves extract. The highest flavonoid content was found in gandaria from maceration method, followed by pohpohan, basil, and lettuce from maceration extracts. In contrast to the TPC, the gandaria traditional leaves extract showed was ranked fifth in TFC content.

Dhital's research (2017) showed that mango leaves as a plant from the same family with gandaria (*Anacardiaceae*), contained flavonoids of 55.63 mg QE/g maceration extract. It means that *Anacardiaceae* family contains high flavonoids as antioxidants. Geographical location, time and climate differences, soil conditions, administration of pesticides or herbicides in plants are factors that can influence vegetable nutrients, antioxidants, and flavonoids (Andarwulan *et al*. 2010).

Overall, the TPC and TFC in maceration leaves extract had higher values than the traditional extraction. In the maceration method the sample or material to be extracted is soaked

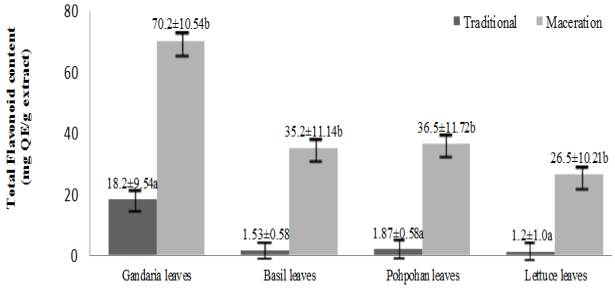

Figure 2. Total flavonoid content of leaves extract. Different letters (a-b) for each leaves are significantly different ($p<0.05$)

in a closed container using a solvent and allowed to remain at room temperature with an adequate agitation. This process aims to soften or break down the plant cell walls so that phytochemical compounds can dissolve in solvents. During the immersion process, the cell wall and cell membrane of the sample will break because of the different pressure between the inside and outside of the cell and the content of secondary metabolites in the cell plasma will be dissolved in the solvent. In addition, the time of maceration can be adjusted according to the research purpose thus able to yield better result (Ibrahim & Marham 2013).

The selection of solvents in the extraction process will also affect the effectiveness of the traceability based on solubility of natural material compounds in the solvent (Ibrahim & Marham 2013). Solvents that have the same polarity as solutes dissolve the substance better. Water is one of the solvents that is often used in extraction processes with high polarity (Altemimi *et al*. 2017). High polarity solvents will also attract phytochemical components that also have high polarity levels such as phenols and flavonoids (Harborne 1987; Robinson 1995; Stankovic 2011).

The IC_{50} value of gandaria, basil, pohpohan, and lettuce leaves showed that all maceration leaves extracts had higher antioxidant activity compared to traditional leaves extracts, especially the gandaria leaves extract showed the highest value of antioxidant activity (Table 2). Vitamin C as a standard in this research had an IC_{50} value of 10 µg/ml. A low value of antioxidant activity could be caused by the content of bioactive components of each sample with different extraction, and crude extract without partitioning used in this research is probably cause antioxidant activity to be of low value (Rahman *et al*. 2017). Andina and Musfirah (2017) reported that the antioxidant activity of gandaria leaves extract with maceration was high in antioxidant activity (IC_{50} 55.83 µg/ml). It means that gandaria showed the highest antioxidant potential.

Our results showed that antioxidant activity in both extraction were significantly different for each leaves extracts ($p<0.05$). The results showed that antioxidant activity also significantly different between each of leaves extracts. It means that the extraction method and leaves affected the antioxidant activity of gandaria, basil, pohpohan, and lettuce leaves. Manggala *et al*. (2017) reported that the antioxidant activity of mango leaves extract with maceration method (*Mangifera indica*) was higher (IC_{50} 5.09 µg/ml) compared to the extract from reflux method (IC_{50} 8.35 µg/ml).

Analysis of α-amylase inhibitory using acarbose as a positive control, showed very high inhibitory activity with IC_{50} value of 0.00045 µg/ml. Our results showed that gandaria and basil leaves extract with maceration have high α-amylase inhibitory activity (IC_{50} 60 and 70 µg/ml) compared to other samples. Inhibitory action for α-amylase enzyme in both extraction were significantly different for each leaves extracts ($p<0.05$). The results showed that α-amylase inhibitory activity also significantly different between each of the leaves species extracts. It means that the extraction methods and leaves affect the inhibitory activity of gandaria, basil, pohpohan, and lettuce leaves. Hence, we found that the leave extract which showed the highest α-amylase inhibitory activity is the one that has the highest antioxidant activity, TPC, and TFC value. The extract was gandaria leaves with maceration extraction method (Table 3).

Bhuvaneshwari *et al*. (2014) showed that mature and tender mango leaves extracted by reflux had an IC_{50} values of 22.01 and 35.73 µg/ml, respectively. It showed that the *Anacardiaceae* family has very high of α-amylase inhibitory activity. El-Beshbishy and Bahashwan (2011) reported that the inhibitory activity of α-amylase enzyme in basil extract was indicated by IC_{50} value of 42.50 mg/ml. The inhibitory activity is probably from the phenolic and flavonoid content of basil. Rahayuningsih (2014) reported that the

Table 2. Antioxidant activity of leaves extract

Leaves extract	IC_{50} (µg/ml)	
	Traditional	Maceration
Vitamin C (10 µg/ml)**		
Gandaria (*B. macrophylla*)	649[a]	35[b]
Basil (*O. africanum*)	>2,000[a]	670[b]
Pohpohan (*P. melastomoides*)	>2,000[a]	250[b]
Lettuce (*L. sativa*)	>2,000[a]	183[b]

* Different letters (a-b) for each leaves are significantly different ($p<0.05$)

** Vitamin C as standar

Table 3. α-amylase inhibitory activity of leaves extract

Leaves extract	IC_{50} (µg/ml)	
	Traditional	Maceration
Acarbose (0.00045 µg/ml)**		
Gandaria (*B. macrophylla*)	163[a]	60[b]
Basil (*O. africanum*)	>1,000[a]	70[b]
Pohpohan (*P. melastomoides*)	>1,000[a]	105[b]
Lettuce (*L. sativa*)	>1,000[a]	117[b]

*Different letters (a-b) for each leaves are significantly different ($p<0.05$)
**Acarbose as positive control

flavonoid content pohpohan (*Pilea trinervia*) leaves extract had an anti-hyperglycemia effect as indicated by glucose tolerance test in glucose induced diabetic rats. Lactucaxanthin (Lxn) of lettuce (*Lactuca sativa*) function as an inhibitor of carbohydrate digestive enzymes. Gopal *et al* (2017), reported that the inhibitory activity of the α-amylase enzyme from Lxn had an IC_{50} value of 434.5 µg/ml.

Maintaining blood glucose levels effectively can be obtained from natural sources such as from the bioactive compounds in vegetables that can function as α-amylase inhibitor. Natural sources inhibitors, such as the one coming from the vegetables commonly consumed are highly recommended because they tend to not cause side effects when consumed (Tundis *et al*. 2010). In addition, Samudra *et al*. (2015) stated that secondary metabolites have high ability to inhibit the activity of the α-amylase enzyme compared to the primary metabolites.

Gu *et al*. (2015) stated that phenolic acids and flavonoids can inhibit the activity of the α-amylase enzyme. Phenolic acids and flavonoids will form covalent bonds with α-amylase into quinones or lactones and react with nucleophilic groups, this causes changes in the activity of the α-amylase enzyme (Tadera *et al*. 2006). Reaction of phenolic compounds and proteins be able to inhibit enzymatic activity including α-amylase enzymes (Yanishlieva-Maslarova 2001). Polyphenols will form complexes with starch so that the α-amylase enzyme cannot recognize the substrate (Himmah & Handayani 2012).

The effect of flavonoids on the α-amylase enzyme using enzymatic kinetic and fluorescence spectroscopy shows that flavonoids will form complexes with the α-amylase enzyme. Flavonoids will be bound to the α-amylase enzyme in a hydrophobic (Yuan *et al*. 2014). Flavonoids are also able to inhibit α-amylase activity because they have the ability to form quinones with 4-oxopyrane (Adisakwattana & Chanathong 2011). Flavonoids as antioxidants are able to prevent a decrease of β-pancreatic cells function progressively that caused by oxidative stress. Thus, it can reduce the occurrence of type 2 diabetes and can prevent the complications of diabetes in the long term (Song *et al*. 2005).

Within this research, the amount of Total Dissolve Solid (TDS) in samples with different extractions was not exactly the same thus it can be consider as a weakness in our methodology. However, the difference is due to the fact that different extraction methods will also show different sample forms. Therefore, to minimize this case, the researchers weighed all extracts after the evaporation process before preparing the stock solutions. So that the Total Dissolve Solid (TDS) of the extracts in different methods could still be controlled.

CONCLUSION

Sundanese traditional salad (*Lalapan*) contains various bioactive compounds and identified as potentially rich source of antioxidants and antihyperglycemia. The results of phytochemical screening confirms the presence of polyphenols, saponins, tannins, and flavonoids in gandaria leaves, basil, pohpohan, and lettuce. Extraction methods have a significant effect on TPC, TFC, antioxidant activity, and inhibition of α-amylase enzymes. All maceration extracts have higher of TPC, TFC, antioxidants, and inhibition of α-amylase compared to traditional extracts. The leave extract with the highest TPC, TFC, antioxidants, and inhibition of α-amylase activity is gandaria leaves extract with maceration method.

Based on the results of the research, further analysis is needed regarding bioactive compounds in *Lalapan* with different extraction methods using High Performance Liquid Chromatography (HPLC). Pra-clinical and clinical research is needed to determine the benefits of its bioactive

compounds. Furthermore, *Lalapan* as source of antioxidant can be developed as vegetable-based functional food products for antihyperglycemia.

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AUTHOR DISCLOSURES

The authors have no conflict of interest.

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Fecal Lipid Content, Serum Lipid Profile, and Intra-Abdominal Fat Accumulation in Normal Rats Supplemented with Rice Bran Oil

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ABSTRACT

This study aimed to investigate the mechanism of rice bran oil on altering lipid absorption and blood lipid level in normal rat. Male Sprague Dawley rat age 3 months old, weighted 250–300 g were grouped into three groups: control (aquabidest 1 ml), orlistat (2.16 mg / 200 g body weight), and rice bran oil (1.04 mg γ-oryzanol / 200 g body weight). The intervention was given through oral gavage, daily for 28 days. Indicators observed were growth performance, total cholesterol and serum triglyceride levels, lipid levels in feces, and accumulation of intra-abdominal fat. The results showed that the treatment did not significantly affect body weight gain. Fecal lipid levels of orlistat, rice bran oil and control group respectively were 0.19g; 0.17g and 0.13 g ($p<0.05$), while the percentage of indigestible lipids for orlistat, control and rice bran oil group were 26%, 17% and 13% respectively ($p<0.05$). Total cholesterol and serum triglyceride levels in rice bran oil group were significantly lower than controls. Rice bran oil did not significantly affect the percentage of total intra-abdominal fat and the weight of the heart and kidney ($p>0.05$). The intervention of rice bran oil was shown to reduce blood cholesterol and triglyceride levels in normal mice and did not accumulate intra-abdominal fat. The results suggest that rice bran oil might have an effect on blood lipid regulation but not by preventing lipid absorption.

Keywords: cholesterol, fecal lipids, normal rat, orlistat, rice bran oil

INTRODUCTION

Lipid accumulation especially in abdominal adipose tissue might disrupt lipid metabolism and is often associated with main risk factors of degenerative diseases such as type 2 diabetes mellitus, hypertension, cardiovascular diseases, and dyslipidemia (Golay & Ybarra 2005; Kotchen 2010; Akil & Ahmad 2011; Klop *et al.* 2013). Rice bran has been acknowledged as one of natural remedies for prevention of degenerative diseases, due to its functional components such as antioxidants, dietary fiber, vitamins, essential minerals, and enzyme inhibitors (Hamada 2000). The antioxidant capacity of rice bran is 28.74 AEAC, 15 times more than tomato juice that has 1.87 AEAC (Damayanthi *et al.* 2010).

Oil can be extracted from rice bran which constitutes up to 18–22% of oil. Rice bran oil has slightly sweet taste due to a high content of 4-monomethylsterols (Cicero & Derosa 2005). It is readily sold in supermarket thus can easily be used to substitute palm oil. Rice bran oil contains γ-oryzanol, a mixture of ferulic acid esters of phytosterols and triterpene alcohols, as bioactive compound which has high antioxidant capacity (Damayanthi *et al.* 2004). Previous studies showed that rice bran oil significantly lowered total cholesterol and serum LDL on rats with hypercholesterolemia (Tong *et al.* 2014; Most *et al.* 2005).

It is hypothesized that the serum lipid lowering effect from rice bran oil is due to its γ-oryzanol content, not from its dietary fiber or fatty acid composition, that inhibit lipid absorption in small intestines and excrete it through feces (Ijiri *et al.* 2015). Study by Bhaskaragoud *et al.* (2016) showed that γ-oryzanol from rice bran oil affect

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lipid absorption by decreasing intestinal lipase activity. Thus, the amount of lipid absorbed also reduced. However, a more detailed mechanism of action of the lipase inhibitor of rice bran oil has not been described.

In this study we examined the effect of rice bran oil on inhibition of lipid absorption compared to orlistat (lipase inhibitor drug). To evaluate lipid absorption of rats fed with standard diet with addition of rice brain oil or orlistat, we examined rat's growth performance, fecal lipid content, serum lipid profile, and the intra-abdominal fat accumulation.

METHODS

Design, location, and time

This study was an experimental study using a Randomized Block Design (RBD). Animal intervention was conducted at IPB Veterinary Teaching Hospital, IPB University. Lipid serum analysis, fecal lipid analysis, and organs weighing were performed at Laboratory of Food Analysis and Chemistry of Department of Community Nutrition of IPB University. The study was conducted from April to November 2018. The experiment obtained ethical clearance from Ethics Commission of IPB Veterinary Teaching Hospital, IPB University number 093/KEH/SKE/VIII/2018.

Materials and tools

Twenty-one male Sprague Dawley rats at 3 month of age were purchased from Tropical Biopharmaca Research Center of IPB University. Rice bran oil (Oryza Grace®) was obtained from supermarket in Bogor. Orlistat (Xenical®) was obtained from pharmacy in Bogor. Control diet was from Indonesia Formula Feed (Indonesia Formula Feed). Blood lipid profile analysis used the Cholesterol Reagent Kit (DiaSys) and Triglyceride Reagent Kit (DiaSys). Fecal lipid analysis used the Soxhlet method with hexane as solvent. Body weight and the weight of intra-abdominal fat, kidney, heart, and liver were measured by digital scale.

Procedure

Animals and treatment. The rats were individually housed in wired-bottomed aluminum cage (170x170x240 mm) and provided with water and control diet ad libitum. The room temperature was maintained at 25°C with 12 hours dark and 12 hours light period every day. The adaptation period was 7 days, followed by random assignment of the rats into three groups on day 8: 1. control diet (energy 2,750 kcal/100g, 4% fat, 23% crude protein, 17% digestible crude protein, 5% crude fiber) and 1 ml of aquabidest; 2. control diet and orlistat with dose of 10.8 mg/kg BB; 3. control diet and rice bran oil with dose of 57.6 mg γ-oryzanol/day. Orlistat solution was prepared by diluting 64.8 mg of crushed orlistat tablet into 100 ml of 1% Na-CMC to obtain final orlistat content of 0.0648%. Concentration of orlistat solution given to rat was adjusted weekly according to its body weight. Final volume given to rat was not exceeding 5 ml. Treatment was given daily for 28 days. Aquabidest, orlistat, and rice bran oil were given through oral gavage daily at 9 am. Body weight was measured every week. Feed intake was measured every day. Feed efficiency were calculated as proportion of feed intake to body weight gain. All rats were euthanized under anesthesia. Blood was collected through the jugular vein. The liver, heart, adipose tissues (mesenteric, retroperitoneal, and epididymal), and kidney were collected and weighed. Fecal samples were collected during last 4 days of the experiment.

Serum Lipid Profile Analysis. The level of triglycerides and total cholesterol were measured using reagent kit (DiaSys) according to the manufacturer's procedure.

Determination of lipid content of feces. Fat content of feces were measured using Sohxlet method. Feces from the last four days were dried in the oven, then grinded and homogenized. Homogenized fecal sample for analysis were sampled (2 g/sample) using AOAC Official Method 925.08 sampling method. Hexane was used as solvent and heating process was running for 6 hours. Fat content was calculated as percentage of amount of lipid extracted to sample weight.

Data analysis

Data were expressed as means±SEM. Analysis for body weight difference pre and post intervention was performed using paired sample t-test. Feed intake, organ weight, and serum lipid profile were analyzed using ANOVA. All statistics analyses were performed with SPSS version 22.0 for Windows.

RESULTS AND DISCUSSION

Effect of rice bran oil (RBO) on rat growth performance

The effect of RBO on body weight gain, average calorie intake, feed efficiency and lipid absorption are presented in Table 1. After 28 days, total body weight gain of RBO group was not different from the control group. Although the daily calorie intake between groups did not differ significantly, daily lipid intake of RBO group was significantly higher than other groups. This is resulted from the induction of RBO that contributed to additional lipid intake. Both RBO and Orlistat group have higher fecal lipid content.

In this study we observed that in normal physiology state, RBO will not result in significant body weight gain. This finding is in line with previous study which showed that rats intervened with High Fat Diet (HFD) did not lead to excessive body weight gain when given RBO orally along with the diet (Son *et al.* 2009). Moreover, study in normal rats fed either standard diet or rice bran containing diet showed a similar growth performance (Iijiri *et al.* 2015).

It is known that γ-oryzanol might affect lipid absorption which reflected on the number of fecal lipid content. Statistical test results showed that the Orlistat and RBO group had significantly more fecal lipid content than the control group. As it is expected from Orlistat which known to act as inhibitor of the pancreatic lipase enzyme, the increase in fecal lipid content of RBO group is thought to have different mechanism than Orlistat. The average percentage of absorbed lipid for control, orlistat, and RBO group is 83.49±0.71%, 73.75±1.34%, and 86.96±0.27% respectively. This data show that the amount of lipid absorbed for RBO group was similar to the control group. Thus, unlike orlistat, the lipase enzyme in RBO group is not inhibited. Lipid absorption inhibition is more prominent to rats when fed with whole rice bran oil, so it might be due to the effect of insoluble fiber included in rice bran than γ-oryzanol (Iijiri *et al.* 2015). Possible explanation for higher fecal lipid content might result from higher lipid intake. Therefore, in this study RBO is not effective to prevent lipid absorption in the intestines.

Effect of RBO on serum lipid profile

The effect of RBO on the total cholesterol and triglyceride level are presented in Table 2. RBO has significantly lower total cholesterol and triglycerides level than the control. Moreover, the low level was comparable to Orlistat treatment. This blood lipid lowering effect might be due to γ-oryzanol content in RBO.

It is known that γ-oryzanol is able to inhibit cholesterol and triglycerides absorption in the intestines and excrete it into feces (Rong *et al.* 1997; Wilson *et al.* 2007). However, as mentioned previously, we found that RBO group showed to have higher percentage of

Table 1. Growth performance and lipid absorption

Parameter	Control (n=5)	Orlistat (n=5)	RBO (n=5)
Growth Performance			
Body weight gain (g/28 days)	25.20±8.40[a]	6.40± 9.54[a]	20.00±8.23[a]
Calorie intake (kcal/day)	550.00±81.10[a]	521.30±51.60[a]	491.20±72.40[a]
Feed efficiency	0.04±0.01[a]	0.01±0.02[a]	0.04±0.02[a]
Lipid Absorption			
Lipid intake (g/day)	0.80±0.33[a]	0.76±0.36[a]	1.29±0.28[b]
Fecal lipid content (g)	0.13±0.01[b]	0.19±0.01[a]	0.17±0.01[a]

Data are expressed as the mean± SEM; Different letters (a and b) indicate significant difference at $p<0.05$ in the one-way ANOVA; RBO: Rice Bran Oil

Table 2. Blood lipid profile

Parameter	Control (n=5)	Orlistat (n=5)	RBO (n=5)
Total Cholesterol (mg/dl)	57.99± 0.99[a]	48.15±0.03[b]	46.89±0.20[b]
Triglyceride (mg/dl)	65.30±0.74[a]	50.96±0.26[b]	53.06±0.43[b]

Data are expressed as the mean±SEM; Different letters (a and b) indicate significant difference at p<0.05 in the one-way ANOVA; RBO: Rice Bran Oil

lipid absorbed compared to control and orlistat. Therefore, assumption that γ-oryzanol prevent lipid absorption is ruled out. While in this study we cannot confirmed the number of cholesterols absorbed, γ-oryzanol can also target HMG-KoA reductase which suppresses mevalonate synthesis (Chithra *et al.* 2015). Reduction in mevalonate will eventually lower cholesterol level in plasma. We assumed the role of γ-oryzanol is more on regulating lipid metabolism instead of inhibiting lipid absorption.

Unlike saturated fat, total fat intake has proven to have no significant association with total cholesterol and triglyceride level (Joy *et al.* 2007). Rice bran oil composed of mostly unsaturated fatty acids (75%) compared to saturated fatty acids (25%). Thus, even though supplementation of RBO increase the total fat intake, effect on blood lipid profile distortion is low. Along these lines, beneficial effect of RBO on improving plasma lipid level can also be seen in normal physiology state.

Effect of RBO on tissue weight

Rats in the RBO group tend to have lower intra-abdominal adipose tissue (1.11±0.45%bw) compare to control (1.41±0.18%bw) and orlistat (1.23±0.24%bw) group as shown in Table 3. However, these differences are not significant. This finding is in line with previous studies which showed that oryzanol is able to significantly decreased epididymal, mesenteric, and peritoneal fat, but not kidney and heart (Wang *et al.* 2015; Iijiri *et al.* 2015; Charkonpunya 2010; Kobayashi *et al.* 2019).

Mechanism on how RBO able to prevent lipid accumulation is still unknown. However, study by Yang *et al.* (2019) found that rice bran can alleviate adiposity by partially reducing the increase of diglycerides and triglycerides in obese rats. Furthermore, study on molecular level shows that RBO down-regulate lipogenic genes, such as Sod1 and Cat, which reduce hepatic level of triacylglycerol (Ahmed *et al.* 2018). This result suggests that RBO has some roles in regulating

Table 3. Effect on tissue weight

Parameter	Control (n=5)	Orlistat (n=5)	RBO (n=5)
Final Body Weight (g)	305.80±7.70[b]	278.40±15.98[a]	290.30±9.35[a]
Total intra-abdominal fat (%bw)	1.41±0.18[a]	1.23±0.24[a]	1.11±0.45[a]
Mesenteric fat (%bw)	0.34±0.08[a]	0.24±0.05[a]	0.28±0.14[a]
Retroperitoneal fat (%bw)	0.34±0.18[a]	0.31±0.14[a]	0.25±0.24[a]
Epididymal fat (%bw)	0.73±0.09[a]	0.68±0.17[a]	0.58±0.25[a]
Kidney (%bw)	0.69±0.04[a]	0.64±0.04[a]	0.68±0.08[a]
Heart (%bw)	0.32±0.02[a]	0.32±0.04[a]	0.30±0.02[a]

Data are expressed as the mean±SEM; Different letters (a and b) indicate significant difference at p<0.05 in the one-way ANOVA; %bw = % body weight; RBO: Rice Bran Oil

adiposity. Further studies are necessary to confirm this mechanism.

CONCLUSION

Beneficial effect of RBO supplementation on blood lipid level in this study does not derived from inhibition of lipid absorption. While the exact mechanism is still unknown, RBO supplementation in normal physiology did not affect both final body weight and tissue weight of intra-abdominal fat, kidney, and heart. This study was performed in rat thus careful interpretation is needed when translating this study result into human subject.

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AUTHOR DISCLOSURES

The authors have no conflict of interest.

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