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Analysis of Serum Ghrelin Levels and BMI in Obese and Non-Obese Subjects

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ABSTRACT

Obesity is excessive body fat and was associated with the importance of metabolic and endocrine problems in somatotropic secretion in functional obesity. Ghrelin is an acylated peptide hormone produced by the stomach, which is a mediator of the growth hormone secretory receptor. The activity of ghrelin stimulates the release of growth hormone, and appetite and stimulates the metabolism of carbohydrates. Circulating ghrelin levels in healthy people increase during fasting and decrease after meals. This study aims to analyze the difference in ghrelin levels among obese and non-obese subjects. A cross-sectional design research was conducted in August 2022. The samples consisted of obese and non-obese subjects based on Body Mass Index (BMI). Ghrelin levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The data were statistically analyzed using the Mann-Whitney and Spearman tests. $p<0.05$ was reported significant. The samples consisted of 80 obese and non-obese subjects, 39 males and 41 females. There was no difference in ghrelin serum levels in the obese group (1.45±2 ng/mL) compared to the non-obese group (0.67±0.25 ng/mL) with $p=0.233$ ($p>0.05$). There was a positive correlation between ghrelin levels and BMI ($r=0.247$). There was no difference in ghrelin levels between the obese group and the non-obese group, and there was a positive correlation between ghrelin levels and BMI. A higher BMI would lead to higher ghrelin levels.

Keywords: Obesity, ghrelin, BMI

INTRODUCTION

Obesity is defined as abnormal or excessive fat accumulation that poses a health risk. This problem has developed into an epidemic with more than 4 million deaths each year as a result of overweight or obesity in 2017. The global prevalence of overweight or obese children and adolescents aged 5-19 years has quadrupled from 4% to 18%. Approximately 13.5% of adults over 18 years are overweight and 28.7% of them are obese (BMI ≥ 25). Based on the National Medium-Term Development Plan/Rencana Pembangunan Jangka Menengah Nasional (RPJMN) 2015-2019, 15.4% of those populations were obese (BMI ≥ 27), 18.8% of children were overweight and 10.8% were obese. Based on the National Health Indicator Survey (SIRKESNAS) in 2016, the obesity rate (BMI ≥ 27) increased to 20.7%, and the obesity rate (BMI ≥ 25) became 33.5%.[1,2]

TThe Body Mass Index (BMI) is a measurement, which has been used since the mid-19th century to identify adults and adolescents who are significantly overweight based on their height. Body weight divided by height is universally expressed in kg/m^2. Body mass index is often considered as an indicator of body fat size; however, it remains a measure of body fat replacement because it measures overweight, not excess fat. Despite this fact, this study shows that BMI is correlated with a more direct measure of body fat.[3,4]

Ghrelin was first described by Kojima *et al*. in December 1999 as an endogenous ligand for the Growth Hormone Secretagogue Receptor (GHSR). Ghrelin is an acylated peptide hormone produced in the stomach, consisting of amino acids and a ligand for GHSR. Ghrelin's main biological activities include stimulating growth hormone release, stimulating appetite, and carbohydrate metabolism. Increased circulating ghrelin levels occur during fasting and decreased ghrelin levels occur after food consumption in healthy people.[5-7]

Ghrelin has two forms, acyl ghrelin (octanoylated) and deacyl-ghrelin (non-octanoylated). Octanoylated ghrelin is required for the catalytic physiology of ghrelin-O-acyltransferase (GOAT), and 20% ghrelin is octanoylated at the 3rd carbon in its acyl form and releases Growth Hormone (GH). Growth hormone-secreting receptors are expressed mainly in

the anterior pituitary, islets of Langerhans, adrenal glands, thyroid, myocardium, arcuate nucleus, hypothalamus, stroma, Ventral Tegmental Area (VTA), raphe nucleus, cortex, and lenticular hippocampus.[8,9]

A study by Tschop *et al*. in 2001 showed that in addition to stimulating GH secretion, ghrelin also induces weight gain by increasing food intake and reducing fat consumption.[10]

Based on this background, the authors aimed to determine the differences in serum ghrelin levels in obese and non-obese subjects.

METHODS

This study used an observational research design with a cross-sectional approach. The study was carried out at the Clinical Pathology Laboratory Installation at Hasanuddin University Hospital, Makassar; sampling and ghrelin levels were measured at the Research Unit of the Faculty of Medicine Hasanuddin University in August 2022. The research population was all Specialist Medical Education Program (MPPDS) students at the Faculty of Hasanuddin University, Makassar who were willing to take part in the research. The research sample is an accessible population, which meets the inclusion criteria. The inclusion criteria were all volunteers who were classified as obese or non-obese subjects and had no history of diabetes mellitus, hypertension, and malignancy. Anthropometric parameters measured to determine BMI in this study were body weight and height. Body mass index was measured by dividing the value of the body weight in kilograms by the square of the height in meters squared. The classification of obese and non-obese groups was based on BMI. Research subjects with BMI > 25 kg/m^2 were classified into the obese group and subjects with BMI < 25 kg/m^2 were classified into the non-obese group.

Serum ghrelin levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method with a sensitivity of 0.01 ng/mL using the Human GHRL (Ghrelin) ELISA kit (Elabscience) and expressed in ng/mL. Data were analyzed using SPSS. The calculation of the frequency distribution and statistical tests were used for statistical analysis in this study. The Kolmogorov-Smirnov test was used to determine the normality of data. The Chi-Square test, unpaired T-test, Mann-Whitney test, and Spearman rho test for the correlation test were then used for statistical analysis. The p-value <0.05 was reported as significant.

This research was carried out after obtaining ethical clearance by considering respect for the subject, beneficence, non-maleficence, and justice from the Health Research Ethics Commission (KEPK) Faculty of Medicine, Hasanuddin University/RSPTN UH/Dr. Wahidin Sudirohusodo Hospital, Makassar with number 436/UN4.6.4.5.31/PP36/2022.

RESULTS AND DISCUSSIONS

The number of samples required in this study was determined based on the purposive sampling technique with the number of samples calculated based on the cross-sectional calculation formula according to Lameshow *et al.* for unpaired numerical sample sizes as follows:

$$n = \frac{2\ (1.645 + 0.842)10\ 2}{6}$$

$$= 34.2\ (35\ \text{samples/group})$$

The minimum number of samples required was 35; the authors involved 40 subjects in the obese group and 40 subjects in the non-obese group, resulting in 80 total subjects.

Table 1. Characteristics of research subjects

Criteria	n (%)	Mean±SD	Median (min–max)
Gender			
Male	39 (48.8)		
Female	41 (51.2)		
Age (years)		26.39±4.5	31 (22–49)
BMI (Kg/m^2)		26.39±4.9	25.05 (16.98–39.5)
Fasting blood glucose (mg/dL)		67.34±16.7	65 (28.1–106.8)
Obese			
Yes	40 (50)		
No	40 (50)		
Ghrelin levels (ng/mL)		1.06±1.5ng/mL	0.7 (0.3–8.25)

Abbrev: BMI: Body Mass Index

The results revealed that 80 research subjects were involved in this study and divided into 2 groups, consisting of 40 obese subjects and 40 non-obese subjects. There were 39 (48.8%) males and 41 (51.2%) females with an age range of 22-49 years. The mean fasting blood glucose level (GDP), BMI, and ghrelin level were 67.34 mg/dL, 26.39 Kg/m2, and 1.06 ng/mL (Table 1).

Based on Table 2, the gender difference according to obesity tested through Chi-Square test results showed no significant difference in gender between obese and non-obese groups with a p-value of 0.823 (p > 0.05). Mann-Whitney test results showed no significant difference in the fasting blood glucose levels with a p-value of 0.988 (p> 0.05), whereas there were significant differences in age (p=0.034) and BMI (p<0.001) (p <0.05). The median and mean were used in the table to determine the mean weight in each group to compare the obese and non-obese groups.

Based on Table 3, the mean ghrelin levels in the non-obese and obese groups were 0.67 ng/mL and 1.45 ng/mL, respectively. However, Mann-Whitney test results showed no significant difference in mean ghrelin levels between obese and non-obese groups with a p-value of 0.233 (p > 0.05).

Based on Table 4, Spearman rho correlation test results showed a significant correlation between ghrelin levels and BMI with a p-value of 0.027 (p <0.05) and a correlation strength of 0.247 (weak correlation).

Table 4. Correlation between serum ghrelin levels and BMI

		Ghrelin
BMI (kg/m^2)	Correlation coefficient	0.247
	p	0.027
	n	80

Abbrev: BMI: Body Mass Index

This study used a cross-sectional research design involving 80 research subjects who met the inclusion and exclusion criteria. The characteristics of the research subjects in Table 1 show that the subjects consisted of 39 (48.8%) males and 41 (51.2%) females. This was in line with a study by Corfe and Shepherd, which found that the prevalence of obesity was higher in females than males.[11,12]

Table 2 explains the differences in characteristics of gender, age, fasting blood glucose levels, and BMI values between the obese and non-obese groups. There were no significant differences in gender with a p-value > 0.05. There was no significant difference in age, fasting blood glucose levels, and BMI values between obese and non-obese groups with a p-value > 0.05. In addition, there was a significant difference in BMI and age between obese and non-obese groups with a p-value <0.05. This finding was in accordance with a study by Vittal *et al.*, which found a gradual increase in the magnitude of BMI along with increasing age in several decades.[13]

Table 2. Different characteristics between obese and non-obese group

Variable	Non-Obese n(%)	Non-Obese Mean±SD Median (min-max)	Obese n(%)	Obese Mean±SD Median (min-max)	p
Gender					
Male	20		19		0.823*
Female	20		21		
Age (years)		30.58±4.8 30 (22-49)		32.4±4.1 32 (26–42)	0.034**
Fasting blood glucose (mg/dL)		67.84±17.8 65.15 (28.1–103.9)		66.83±15.7 64.95(35–106.8)	0.988**
BMI (Kg/m^2)		22.31±1.58 22.65 (16.98 – 24.4)		30.47±3.6 29.79 (25.7–39.5)	< 0.001**

Abbrev: BM: Body Mass Index, *Chi-Square test, **Mann-Whitney test

Table 3. The difference in ghrelin levels between obese and non-obese group

Variable	Non-Obese Mean±SD Median (min-max)	Obese Mean±SD Median (min-max)	p
Ghrelin levels (ng/mL)	0.67±0.25 ng/mL 0.71 (0.3 – 1.09)	1.45±2 ng/mL 0.70 (0.31 – 8.25)	0.233*

*Mann-Whitney test

In Table 3 there was no difference in ghrelin levels between the obese group (1.45±2 ng/mL) and the non-obese group (0.67±0.25 ng/mL) with p=0.233 (p> 0.05). These results were in line with a study by Omran *et al*., which found no significant relationship between fasting serum ghrelin and obesity. Studies in rodents showed that ghrelin plays an important role in signaling to the hypothalamic center to regulate food and a hypothesis by Tscho¨p *et al*., found lower plasma ghrelin levels in obese subjects compared to non-obese subjects. In addition, plasma ghrelin concentrations are significantly lower in Pima Indians than in Caucasians. Ghrelin secretion in physiological and pathological conditions remains unknown and remains a matter of controversy. Ghrelin concentration in circulation is inversely proportional to BMI; ghrelin secretion will increase in anorexia (loss of appetite) and cachexia (weight loss due to chronic disease), but decreases in obesity and returns to normal when ideal body weight is achieved. Ghrelin levels will also decrease in fasting obese people, which may be a compensatory response to maintaining energy balance.[5,10,14]

Based on Table 4, Spearman rho correlation test results showed a positive correlation between ghrelin levels and BMI with r=0.247; p <0.05 and a correlation strength of 0.247 (weak correlation). These results were in line with research by Amin *et al*., which found a significant positive correlation between ghrelin and BMI across the study population.

Obesity is a major public health problem, which is defined when the BMI is greater than 25 kg. The prevalence of obesity in the United States is estimated at 31%. More than 15% of the population in European countries such as England, Germany, and Poland are obese. Body mass index is defined as body weight in kilograms divided by the square of height in meters (kg/m^2).[15,16]

CONCLUSIONS AND SUGGESTIONS

There was no significant difference between ghrelin levels in the obese group and the non-obese group. There was a positive correlation between ghrelin levels and BMI, which indicated that a higher BMI would possibly lead to higher ghrelin levels.

The examination of ghrelin levels in a wider range of subjects by distinguishing obese 2, obese 1, and non-obese subjects. Examination of ghrelin levels about the disease in non-obese with diabetes and obese with diabetes.

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