CORRELATION BETWEEN GHRELINE AND BONE MASS DENSITY IN MENOPAUSE WOMEN

[1]Fakhrurrazi, [1]M. Fidel Ganis Siregar, [1]M. Oky Prabudi, [1]Sanusi Piliang, [1]T.M Ichsan Ibrahim, [1]Sarah Dina
[1]Departmen of Obstetrics and Gynecology,
[1]Faculty of Medicine, Universitas Sumatera Utara.

***Abstract* :** This research is a descriptive study with a case approach to determine the correlation of the value of ghrelin with bone density in menopausal women. This research was carried out at Obstetrics and Gynecology SMF at H. Adam Mlaik General Hospital. The USU Department of Obstetrics and Gynecology was conducted in December 2018 until February 2019. Samples that met the inclusion and exclusion criteria were then drawn for blood and examined for serum Ghrelin levels and Bone Mass Density (BMD) examination.) Then a statistical correlation analysis was performed to assess the relationship between levels of ghreline and Bone Mass Density for Menopause women. From the results of the study, most of the research subjects belong to the classification of osteopenia as many as 23 people (76.7%), in this study there were also 2 (6.7%) subjects who had osteoporosis and the rest in normal conditions as many as 5 people (16.7%). there was a significant correlation between ghrelin levels and bone mass density (r = - 0.397; p = 0.030) with a correlation with r count for bone mass density greater than r table and positive This study concludes that there is a significant correlation between ghrelin levels and bone mass density (r = - 0.397; p = 0.030) with a negative correlation with r count for bone mass density greater than r table.

Keywords: Menopause, Ghreline, Bone Mass Density.

I. INTRODUCTION

Based on data from Central Statistics Agency (2005) the number of women living in premenopausal age (40-50 years) approach 13,543 million, while the number of women > 50 years old entering menopause is estimated to increase significantly. More susceptible changes occur during menopause namely osteoporosis. Usually within 5 to 10 years after menopause, osteoporosis can occur. In these years, more osteoporosis occurs in the wrists of the thighs and also in the spine, causing back pain.[1]

According to data from International Osteoporosis Foundation (IOF) it is estimated that the prevalence of osteoporosis is 28.7% of men over 50 years and 32.3% of women over 50 years. The process of menopause is characterized by a decrease in estrogen levels associated with a decrease in bone mass more rapidly thereby increasing the risk of osteoporosis.[2,3] The incidence of osteoporosis is also associated with an increased incidence of fractures due to decreased bone mineral density. Bone Mineral Density (BMD) can be used for prognosis and follow-up in treatment of osteoporosis. Treatment is said to fail if BMD has decreased or there is a fracture. According to WHO in 1994, bone mass density (BMD) was measured based on T scores, and was said to be osteoporosis when T scores were less than -2.5.[3]

Obesity and Body Mass Index (BMI) have a relationship with the risk of fracture. Body weight, inversely related to bone loss and bone remodeling in postmenopausal women. Other studies have shown a positive relationship between bone mass density (BMD) and fat mass (FM). This can be due to biochemical signals of modified adipose tissue such as leptin, adiponectin, resistin, and ghrelin. This provides the hypothesis that the effect of weight gain with BMD might be mediated by the effect of adipokines on bone remodeling.[4]

Ghrelin is an appetite hormone that is thought to affect the relationship between bone mass and fat. The function of chondrocytes has been investigated to synthesize and secrete ghrelin in human cells and rat cartilage. In addition, administration of ghrelin can increase osteoblast proliferation in vitro. In a recent study of adolescents with anorexia nervosa, ghrelin was found to be an important predictor of changes in the spine and bone mass throughout the body.[4,5]

Recent research shows that Ghrelin can be produced by osteoblasts, and Ghrelin receptors (GHS-R) are detected in rat and human bone cells. Although Ghrelin has a positive effect on the proliferation and differentiation of osteoblasts in vitro in vivo studies, in animals has shown no relationship with bone mass in

mice or a positive correlation in mice with an increase in osteoblast-like cell numbers, expression of osteoblast differentiation markers, and bone mass density. Findings from clinical studies in humans, where bone mass density has been evaluated using dual-energy X-ray absorptiometry (DEXA), are also contradictory in cohorts with large samples.[6]

Previous studies reported the expression of Growth Hormone Secretagogue Receptor 1a (GHS-R1a) in mice and showed that ghrelin stimulates cell proliferation and differentiation. In addition, ghrelin also increases Bone Mass Density in both normal mice and GH deficiency. Based on these observations, it can be concluded that ghrelin directly promotes bone formation mediated by GHS-R1a in vitro and in vivo.[7]

II. RESEARCH METHODOLOGY

This research is an analytic study with a case series design on 22 samples of menopausal women who came to RSUP Adam Malik Medan and Setiabudi Hospital Medan and fulfilling the inclusion criteria which is willing to participate in the study and have signed a willingness form, do not undergo menstruation minimum in > 12 consecutive months, do not have history of fracture or osteoporosis, do not have diabetes mellitus, hypertension, heart abnormalities, rheumatoid, or kidney abnormalities, do not undergo ovarian removal / early menopause surgery, do not use hormonal contraception or hormone replacement treatment, do not suffer from malignant diseases, do not have habit of drinking alcohol or smoking, do not use anti osteoporosis agents; and exclusion criteria namely damaged blood serum; from January 2019 until the sample is fulfilled.

After obtaining approval from the ethics commission to conduct research, the research began by conducting interviews and filling out data sheets. After the subject data was obtained, a vacuum tube was taken and the identity of the research subject was taken, followed by a 3 cc blood draw from the mediana cubiti vein, and inserted into a vacuum tube for further delivery to the Integrated Laboratory of Faculty of Medicine, University of North Sumatra.

The blood from the vacuum tube is then transferred into the serum separator tube (according to the identity of the study subjects) and allowed to freeze in 30 minutes at room temperature before being centrifuged for 5 minutes with 5000 x g. Serum separation and examination is carried out as soon as possible and stored at 2-8 ° C. Avoid the thawing-freezing cycle. Further tests were carried out using the Enzyme Linked Immunosorbent Assay (ELISA) method through several stages.

Patients are welcome to follow the bone density measurement procedure using the DXA method at Setia Budi Hospital, Medan. After obtaining laboratory results and DXA interpretation, the data is tabulated and then analyzed statistically.

Data will be analyzed descriptively to see the frequency distribution of the studied variable. The mean difference between variables was tested by T-test and ANOVA test. The relationship between characteristic variables with bone density was tested by Fisher-exact test, then analyzed to see the correlation of serum ghrelin levels to bone density with Pearson correlation test with a significance of $p < 0.05$. The confidence interval of this study was 95%.

III. RESULTS AND DISCUSSION

A total of 30 research subjects were examined and serum Ghrelin levels were examined at the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra and Bone Mass Density (BMD) examination at the Setia Budi Hospital Medan. The results of the study will be described as follows

Characteristics of research subjects based on age, BMI, age at menopause, waist to hip ratio and osteoporosis classification

Table 1. Characteristics of research subjects based on age, BMI, age at menopause, waist to hip ratio and osteoporosis classification

Characteristics	n	%
Age		
45-55 years old	2	6.7
>55 years old	28	93.3
BMI		
Underweight	1	3.3
Normal	10	33.3
Overweight	8	26.7
Obese	11	36.7
Menopause Age		
Early Menopause	13	43.3
Normal	17	56.7
Waist to Hip Ratio		

Normal	13	43.3
Obesitas	17	36.7
Osteoporosis classification		
Normal	5	16.7
Osteopeni	23	76.7
Osteoporosis	2	6.7
Total	**30**	**100%**

Mean Ghrelin Levels Based on Characteristics

Table 2. Mean Ghrelin Levels Based on Characteristics

Characteristics	Category	Ghrelin Level				
		Mean	Median	SD	Min	Max
Age	> 50 y.o.	1.88	1.81	0.39	1.19	2.78
Menopause Age	Early	1.74	1.72	0.28	1.19	2.32
	Average	1.98	2.02	0.43	1.36	2.78
BMI	Normal	2.02	2.04	0.48	1.36	2.78
	Overweight	1.89	1.89	0.20	1.64	2.30
	Obese	1.73	1.63	0.38	1.19	2.32
Bone Density Status	Normal	1.79	1.80	0.24	1.51	2.11
	Osteopeni	1.94	1.86	0.39	1.36	2.78
	Osteoporosis	1.30	1.30	0.15	1.19	1.40
Menopause Duration	1-2 tahun	2.06	2.06	0.06	2.02	2.11
	3-4 tahun	2.67	2.67	0.14	2.57	2.78
	>4 tahun	1.79	1.76	0.33	1.19	2.53
Waist to hip ratio	Good acceptable	1.90	1.80	0.47	1.36	2.78
	Average	1.96	1.89	0.39	1.58	2.57
	High unacceptable	183	1.80	0.30	1.37	2.30
	Extreme unacceptable	1.77	1.72	0.46	1.19	2.32

Correlation of Risk Factors Frequency Based on Bone Density Status of Research Subjects.

Table 3. Age Distribution based on Bone Density Status of Research Subjects

Age	**Bone Density Status**							
	Normal		Osteopeni		osteoporosis		Total	
	n	%	N	%	n	%	n	%
<=50 years old	0	.0%	0	.0%	0	.0%	0	.0%
> 50 years old	5	100.0%	23	100.0%	2	100.0%	30	100.0%
Total	5	100.0%	23	100.0%	2	100.0%	30	100.0%

Table 4. Menopause Age Distribution based on Bone Density Status of Research Subjects

Menopause Age	**Bone Density Status**							
	Normal		Osteopeni		osteoporosis		Total	
	N	%	n	%	n	%	n	%
Early	1	20.0%	11	47.8%	1	50.0%	13	43.3%
Average	4	80.0%	12	52.2%	1	50.0%	17	56.7%
Total	5	100.0%	23	100.0%	2	100.0%	30	100.0%

Table 5. BMI distribution based on Bone Subject Density Status

BMI	**Bone Density Status**							
	Normal		Osteopeni		osteoporosis		Total	
	N	%	n	%	n	%	n	%
Underweight	1	20.0%	0	.0%	0	.0%	1	3.3%
Normal	1	20.0%	9	39.1%	0	.0%	10	33.3%
Overweight	1	20.0%	7	30.4%	0	.0%	8	26.7%
Obese	2	40.0%	7	30.4%	2	100.0%	11	36.7%
Total	5	100.0%	23	100.0%	2	100.0%	30	100.0%

In this research, 23 of the 30 study subjects had osteopenia, 12 of them experienced menopause at an average age. A total of 52.2% of the study sample with an average age of first menopause had osteopenia. 30.4% of the overweight and obese body mass index group had osteopenia and 2 of the obese patients had osteoporosis.

Can be seen from the body mass index, there are 1 (3.3%) research subjects that are classified as underweight, 10 people (33.3%) with a normal body mass index, most of the study subjects came from the obese body mass index group of 11 people (36.7%) and the rest came from the overweight group (26.7%).

Further studies of Sony A et al (2011), stated that the L2-L4 lumbar vertebra T-score had a significant difference between the normal body mass index and overweight categories ($p < 0.05$). This study concludes that body mass index can be the strongest predictor of bone density levels. Body weight, inversely related to bone loss and bone remodeling in postmenopausal women. Postmenopausal women with low body mass index are more prone to osteopenia, and osteoporosis.[8] Body mass index is also used as an important marker to prevent osteoporosis.[5] Purnell, 2003 in his research stated the relationship of ghrelin with body mass index ($r = -0.78$; $p = 0.04$) with a negative correlation coefficient. This data shows the higher the BMI number, the lower the plasma ghrelin level.[4]

Based on the age of menopause, the most research subjects belong to the average menopause age, which is as many as 17 people (56.7%) and the rest experience early menopause as many as 13 people (43.3%). The results of Harris M et al (2015), showed a significant relationship between the duration of menopause and decreased bone density especially in the neck of the femur compared to the spinal lumbar.[9]

Based on the Waist to Hip Ratio category, 13 subjects were classified as normal and 17 were obese. Waist size is inversely proportional to bone loss and bone remodeling in postmenopausal women. Purnell, 2003 in his research stated that there was a relationship between central obesity which was valued with waist to hip ratio with bone density ($r = -0.32$; $p = 0.01$) with negative correlation coefficient. This data shows the higher the waist to hip ratio, the lower the bone density level.[4]

Most of the research subjects were classified as osteopenia, as many as 23 people (76.7%), in this study there were also 2 (6.7%) subjects who had osteoporosis and the rest under normal conditions were 5 people (16.7%). Mpalaris et al. In 2016, measuring serum Ghrelin and Adiponectin and bone mass in the spine and neck of the femur of 84 postmenopausal women found 30 (35.7%) women diagnosed with osteoporosis and 54 (64.2%) women diagnosed with osteopenia.[10]

Correlation of Serum Ghrelin Levels with Bone Mass Density

Table 6. Correlation of Serum Ghrelin Levels with Bone Mass Density

	Mean	SD	Median	Min	Max	r	p
Bone Mass Density	0.800	±0.097	0.807	0.539	0.964	- 0.397	0.030
Ghrelin	1.87	±0.38	1.80	1.19	2.78		

* Pearson Correlation Test

From the results of the resarch, it was found that there was a weak negative correlation between increased levels of ghrelin with a decrease in bone mass density ($r = - 0.397$; $p = 0.030$) and corroborated by a statistically significant test.

This is in line with research conducted by Mpalaris et al in 2016 involving 84 postmenopausal women found 30 women diagnosed with osteoporosis and 54 people diagnosed with osteopenia. This study measured serum Ghrelin and Adiponectin and bone mass in the spine and neck of the femur, and found no significant relationship between Ghrelin with bone mass density ($p = 0.490$).[10] However, this research was a cross-sectional study. so they are susceptible to confounding factors and cannot support a causal relationship. The study of Jurimae et al also showed similar results. The results of this study show similarities in studies examining the effects of grhelin in healthy postmenopausal women. Ghrelin plasma concentrations are related to total BMD values ($r = 0.959$; $p = 0.0001$), and are related to other factors such as body composition, hormones and insulin resistance values.[11] Ghrelin is thought to increase osteoblast replication, osteoblast expression of certain genes, differentiation of osteoblast markers, and bone mineral density (BMD).[10] As well as in line with Carasso et al's 2014 study which showed a decrease in plasma concentration of ghrelin postoperative gastrectomy, associated with a decrease in total bone mineral density.[3]

IV. CONCLUSION

The lowest Ghreline level was found in the obese group, the group with osteopenia bone density status, menopause duration between 3-4 years and weight hip ratio in the average group. Osteoporosis bone density status based on BMI was higher in obese subjects compared to normoweight and osteopenia bone density status based on BMI was more common in overweight and obesity research subjects.

V. ACKNOWLEDGEMENT

The researcher show gratitude for all those who support and assist the implementation of this, and also the research samples who participated in this research. Given the diverse role of ghrelin in life. The results of this study are expected to be ghrelin examination can be used as an alternative examination together with DXA to determine bone density in anticipation of osteoporosis so that life expectancy and quality of life of menopausal women can improve. So it can do screening for menopausal women by using ghrelin to predict the possibility of osteoporosis, but after conducting diagnostic research studies on levels of ghrelin with bone mass density.

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