An Investigation of Serum Ghrelin Concentration and its Relationship with Bone Mineral Density in Healthy Postmenopausal Women over 55 Years in Tabriz in 2013

Haleh Barmaki¹, Faranak Kazerouni², Ali Rahimipour³, Hooshang Amirrasouli, Ali Rahimipour ², Hooshang Amirrasouli, Akbar Ali Asgharzadeh ³, Atefeh Talebi ⁴

¹Senior in Biochemistry, Department of Laboratory Medicine, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
²Ph.D. in Biochemistry, Department of Laboratory Medicine, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
³Ph.D. Endocrinologist, Bone Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
⁴Ms in Biostatistics, Department of Biostatistics, Faculty Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Postmenopausal women are the main population who are at risk of osteoporosis. Ghrelin hormone is a 28-amino-acid peptide secreted in gastrointestinal tract, and primarily in stomach. Several effects are attributed to this hormone which its effects on increasing bone mineral density (BMD) have been taken more attention recently. The purpose of this study was to determine serum ghrelin concentration and its relationship with bone mineral density in healthy postmenopausal women over 55 years.

Methods: This study was conducted in 2013 on 80 postmenopausal women over 55 years who referred to the Bone Densitometry Center of Sina Hospital in Tabriz (Iran). Variables such as age, height, weight, waist-to-hip ratio, menopausal status, experience of disease and medications were recorded. Then, the serum alkaline phosphatase by photometric method, serum ghrelin levels by ELISA, and the status of bone density by using Dual-energy X-Ray Absorptiometry (DXA) in the second to fourth lumbar spine (L2, L4) and femoral neck (FN) were measured. For statistical analysis, Pearson correlation, logistic regression, post hoc test, and one way ANOVA were used.

Results: Serum ghrelin levels showed a significant relationship only with the lumbar spine BMD (BMDLS) (r = 0.23, P = 0.04). Average levels of ghrelin in three age groups of 55-64, 65-74, 75-84 years showed a significant difference (P = 0.04). With increasing age, there was a significant increase in the lumbar spine BMD (P = 0.04). Logistic regression analysis showed a significant relationship between the variables waist-to-hip with femoral neck BMD (OR = 1.24) and statistically significant relationship between variable waist-to-hip with BMD of the lumbar spine (OR = 0.58) and ghrelin variable with BMD of the lumbar spine (OR = 0.018) were found.

Conclusions: The findings of the study showed that serum ghrelin concentration in postmenopausal women with osteoporosis is reduced. In this study, a significant relationship between ghrelin and BMD in the lumbar spine was observed which was in consistent with some other similar studies.

KEYWORDS: Ghrelin, osteoporosis, bone density, osteopenia

1. INTRODUCTION

1.1. Background

Osteoporosis is a disease characterized by decreasing bone mass density and loss of bone microstructure quality which decrease the mechanical strength of bone, increase fracture risk, and their vulnerability to impacts. Low bone mass density occurs slowly and gradually, and often its symptoms does not identified until the first fracture happens (1-3).

Today, osteoporosis is recognized as a major health problem in society and fractures resulting from osteoporosis can impose enormous and irreparable financial and physical damage to society and patients and if untreated, serious consequences will follow (4, 5). The bone mass in women in all age groups is considerably less than that in men in the same age and race (6). Peak bone mass in both sexes will result up to the age 30 and then with increasing age, bone density will decrease in both sexes. The rate of decline after age 40 is less than 1%, about 2% after postmenopausal, and the rate of decline in six years after postmenopausal will reach to the limit of 39% in

Corresponding Author: Faranak Kazerouni, Ph.D. in Biochemistry, Department of Laboratory Medicine, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email:Fk_kazerouni@yahoo.com
year (7). Over 20 years after menopause, 50% of trabecular BMD and 30% of cortical BMD will lose. Women are 8 times more than men are at risk of osteoporosis. The disease affects more than half of women over age 50 (8). So far, about 200 million women around the world suffer from this disease (9). The results of a study showed that 10 million people over the world have osteoporosis and 34 million suffer from loss of bone density. By 2020, approximately 61 million people worldwide have osteoporosis or low bone mass (10). The risk of dying from this disease during the life of a woman is equal to probability of mortality from breast cancer and about four times more likely to die from uterine cancer (11, 12). A danger which threatens the health of a 50 years old woman with femoral neck fracture due to osteoporosis is higher than the cumulative risk of cancer of breast, ovary, and endometrium (13). Menopausal women with osteoporosis are more important because they spend one-third of their life in terms of decreased bone mass and increased fracture risk due to osteoporosis and reduction rate in bone density is very high in the first few years of menopause (13). In a study conducted on 197,848 postmenopausal women from five different races in America, it was shown that about 20% of women of every race at the age of 80 have osteoporosis (14). Another study recently conducted on 256 postmenopausal women in Saudi Arabia, in 74% of the women at lumbar spine and in 59% of them at femoral neck there was the increasing risk of fractures due to osteoporosis (15). In addition, the economic costs of osteoporosis are staggering. In the United States of America alone, direct costs of fractures due to osteoporosis varied from $12 billion to $18 billion and indirect costs especially those that result from reducing economic efficiency should add to total costs of fracture care and these costs will increase to double or triple in the coming decades, because prevalence of osteoporosis is continuing to escalate (16). The annual costs of fractures in America and England are estimated about 1.7 billion pounds and $18 billion respectively (17). The findings of a national program of prevention, diagnosis, and treatment of osteoporosis in Iran show that 70% of women and 50% of men over 50 suffer from osteoporosis and osteopenia (18). The results of study conducted on people of 60-69 years old living in Tehran showed that the prevalence of osteoporosis in lumbar spine in women were 32.4% and in men were 9.4% and peak bone density at all ages of Tehran residents were totally below than standard (19). One notable review in connection with osteoporosis in elder women is studying of serum ghrelin levels. Ghrelin is a hormone that increases food intake, secretion of growth hormone, energy balance, and long-term regulation of body weight (20). Another important role of ghrelin is its effects on bone metabolism (21, 22). Several studies have examined the relationship between the hormone and bone density, so the existence of receptors in osteoblast cells has been demonstrated (21-23). Several hormones are involved in the secretion or inhibition of that (20, 24) including growth hormone, somatostatin, leptin, and insulin which are considered as ghrelin inhibitors. Hypothyroidism is stimulator of ghrelin secretion (20, 24, 25, 26). Several studies indicate a strong correlation between adipose tissue and bone mass density (27, 28). Adipose tissue is the main site of aromatization of androgen to estrogen contributing to increases BMD in both postmenopausal women and men (29). Studies on animal models and postmenopausal women show that estrogen deficiency is concerned with increasing bone resorption and decreasing bone density (30, 31). The relationship between adipose tissue and bone is complex and it is thought that a number of hormones and cytokines modulate osteoclastogenesis by enhancing osteoclast differentiation and hormonal factors associated with adipose tissue, including sex hormone, adiponectin, leptin, and insulin effect on bone density indirectly (32, 33). There are conflicting results on the effect of ghrelin hormone on bone remodeling in humans. In a study carried out by Napoli and his colleagues a significant relationship between ghrelin and the trabecular BMD in women was demonstrated (34). The study of Fukushima also showed a direct relationship between the hormone and bone mineral density. According to studies which showed the relationship between ghrelin hormone and BMD in some people, the present study also aims to determine the relationship between ghrelin hormone and BMD in postmenopausal women in both hip and waist areas. The purpose of this study was to investigate serum ghrelin concentration and its relationship with bone mineral density in healthy postmenopausal women over 55 years. The specific objectives of this study were adjusted as follows:
• To determine the total BMD, femoral neck BMD, and lumbar spine BMD in healthy women over 55 years
• To determine the relationship between serum ghrelin concentration and BMD in healthy women over 55 years
• To determination serum alkaline phosphatase concentration in healthy women over 55 years
• To determine the relationship between serum ghrelin concentration and alkaline phosphatase enzyme in healthy women over 55 years
• To determine demographic characteristics (body mass index, waist-hip-ratio, and age) in healthy women over 55 years
• To determine the relationship between demographic characteristics (body mass index, waist-hip-ratio, and age) in healthy women over 55 years
2. METHODS

2.1. Research Design and Setting

This study was a cross-sectional study conducted in 2013 on 80 postmenopausal women over 55 years who referred to the Bone Densitometry Center of Sina Hospital in Tabriz (Iran) to determine bone mass. Exclusion criteria included the experience of diabetes, kidney, liver, and heart disease, hyperthyroidism, undergoing hormone therapy (including insulin), and experience of malignant disease.

2.2. Measurement Tools and Data Collection

The check list used in this research included demographic characteristics such as age, height, weight, truncal obesity (waist circumference, hip circumference), menopausal status, experience of disease and medications. BMI was calculated and recorded for each individual. Weight was measured by using a digital scale with the accuracy of at least 0.1 kg and capable of being calibrated (manufactured by Beurer, Germany) and height by using a stadiometer with accuracy of at least 0.1 cm and the Broca page. Extra heavy clothes and shoes were removed before measurement. Waist circumference was determined between intercostal margin (margin gear) and iliac crest. Hip circumference was measured at the level of greater trochanter. The BMD of women in lumbar and femoral was measured by LUNAR system DPC-MD model using DEXA technique. Blood samples were taken after 12 hours of fasting. All samples were immediately centrifuged for 10 minutes with rpm 3000 and segregated. The alkaline phosphatase was analyzed on the same day of sample collecting by BT3000 plus auto analyzer and done by Pars azmun kit. Some of the serums were maintained in freezer - 20 °C until the day of testing to test ghrelin. Ghrelin was measured by ELISA method using kits Human Total Ghrelin from Yangpu District made in China (Product Code: E3091Hu).

2.3. Ethical Consideration

This research was approved by the ethics committee of Shahid Beheshti University. Due to the nature of the research, the samples used for this study were of the same blood samples obtained for routine tests based on protocols governing the medical center and if it was a need to prepare new samples, they were collected after full consent of patient and enough explaining for the cause of that.

2.4. Statistical Analyses

Statistical analyzes were done using SPSS-16 and the significance level of less than 0.05 was considered. Statistical techniques used in this study include ANOVA test, independent samples t-test, test, Pearson test, Chi-square test, and Logistic regression analysis techniques.

3. RESULTS

Total 80 women over 55 years were examined in this study; So that 67 (83.8%) were between 55-64 years, 8 (10%) were between 65-74 years, 5 (6.2 %) patients were between 75-84 years. The mean, standard deviation, and characteristics of the candidates are given in Table 1.

The breakdown of body mass index (kilogram per square meter) shows that 2 patients (2.5 %) were underweight, 17 (21.2%) were normal, 41 (51.2 %) were overweight, and 20 (25%) were obese. The frequency of people with osteoporosis were 24 (30%), with osteopenia were 29 patients (36.2%), and healthy individuals were 27 patients (33.8 %).

The correlation coefficient between ghrelin and the various factors studied are shown in Table 2 and as it is considered the correlation coefficient between ghrelin and BMD of the lumbar spine is statistically significant and shows a direct relationship. Average of ghrelin hormone in terms of BMD is shown in Table 3. Ghrelin levels in obese people were less than those in normal BMI and overweight, but revealed no significant differences between age groups (P = 0.31).

Average of ghrelin hormone in terms of bone mass is shown in Table 3. Based on the results, ghrelin levels in people with osteoporosis were less than those in people with osteopenia and healthy people and it was not statistically significant (P = 0.13).

In addition, the average of ghrelin hormone in age group of 65-74 years old was more than those in two other groups and in the age group of 75-84 years was less than those in two other groups and it was statistically significant (P = 0.04) (Table 3).

The mean of BMD was also measured at different ages (Table 4). In this section of findings, the mean of BMD of the lumbar spine was significant and it was shown that differences among these three groups were
statistically significant by using ANOVA test (P = 0.04). Finally, using the Post Hoc test showed significant differences between the age group of 55-64 years and 65-74 years. The results of logistic regression test, showed significant relationship between the dependent variable, femoral neck BMD, and the independent variable of waist-to-hip (P = 0.023, OR = -1.247) (Table 6). In addition, significant relationship between the dependent variable, lumbar spine BMD, and the independent variables, and ghrelin (P = 0.019, OR = 0.018) and waist-to-hip (P = 0.029, OR = -0.582) were observed (Table 6).

Table 1. The mean and standard deviation of studied variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>SD±μ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>0.73±1.57</td>
</tr>
<tr>
<td>Ghrelin (ng/ml)</td>
<td>0.24±2.14</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>0.45±27.52</td>
</tr>
<tr>
<td>WHR</td>
<td>0.007±0.89</td>
</tr>
<tr>
<td>ALP (U/I)</td>
<td>8.09±197.2</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>1.24±71.2</td>
</tr>
<tr>
<td>Femoral Neck BMD (g/cm²)</td>
<td>0.03±0.74</td>
</tr>
<tr>
<td>Lumbar Spine BMD (g/cm²)</td>
<td>0.01±0.85</td>
</tr>
<tr>
<td>Total BMD (g/cm²)</td>
<td>0.42±1.25</td>
</tr>
</tbody>
</table>

Table 2. The correlation coefficient between serum ghrelin hormone and other studied variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age (year)</th>
<th>BMI (Kg/m²)</th>
<th>WHR</th>
<th>ALP (U/I)</th>
<th>Weight (Kg)</th>
<th>Femoral Neck BMD (g/cm²)</th>
<th>Lumbar Spine BMD (g/cm²)</th>
<th>Total BMD (g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.099</td>
<td>0.8</td>
<td>0.91</td>
<td>0.62</td>
<td>0.397</td>
<td>0.906</td>
<td>0.04</td>
<td>0.606</td>
</tr>
<tr>
<td></td>
<td>0.006</td>
<td>-0.01</td>
<td>0.013</td>
<td>-0.056</td>
<td>0.096</td>
<td>-0.013</td>
<td>0.23</td>
<td>-0.059</td>
</tr>
</tbody>
</table>

Table 3. The mean of ghrelin hormone in studied patients based on bone mass, body mass index, and age

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>N (%)</th>
<th>Ghrelin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Density</td>
<td>Osteoporosis</td>
<td>24(0)</td>
<td>1.23±0.13</td>
</tr>
<tr>
<td></td>
<td>Osteopenia</td>
<td>29(36.2)</td>
<td>2.13±0.41</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>27(33.8)</td>
<td>2.97±0.53</td>
</tr>
<tr>
<td>BMI</td>
<td>&lt;18.5</td>
<td>2(2.5)</td>
<td>1.05±0.35</td>
</tr>
<tr>
<td></td>
<td>18.5&lt;=BMI&lt;25</td>
<td>17(21.2)</td>
<td>2.16±0.51</td>
</tr>
<tr>
<td></td>
<td>25&lt;=BMI&lt;30</td>
<td>41(51.2)</td>
<td>2.53±0.4</td>
</tr>
<tr>
<td></td>
<td>BMI&gt;=30</td>
<td>20(25)</td>
<td>1.46±0.24</td>
</tr>
<tr>
<td>Age</td>
<td>55-64</td>
<td>67(83.8)</td>
<td>2.01±0.26</td>
</tr>
<tr>
<td></td>
<td>65-74</td>
<td>8(10)</td>
<td>3.73±0.93</td>
</tr>
<tr>
<td></td>
<td>75-84</td>
<td>5(6.2)</td>
<td>1.02±0.17</td>
</tr>
</tbody>
</table>

Table 4: The mean bone mineral density at different ages

<table>
<thead>
<tr>
<th>Age</th>
<th>BMD lumbar spine Density (g/cm²)</th>
<th>BMD Total (g/cm²)</th>
<th>BMD Femoral Neck (g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55-64</td>
<td>0.88±0.01</td>
<td>1.35±0.5</td>
<td>0.77±0.03</td>
</tr>
<tr>
<td>65-74</td>
<td>0.76±0.06</td>
<td>0.76±0.05</td>
<td>0.64±0.02</td>
</tr>
<tr>
<td>75-84</td>
<td>0.74±0.08</td>
<td>0.67±0.096</td>
<td>0.05±0.05</td>
</tr>
</tbody>
</table>

4. DISCUSSION

The present study was conducted on 80 women with a mean age of (61.5 ± 0.73 years). In this study, the people were divided to three age groups (55-64 years), (65-74 years), and (75-84 years). The mean serum ghrelin in the three groups were (2.01 ± 0.26 ng/ml), (3.73 ± 0.93 ng/ml), and (1.02 ± 0.17 ng/ml) respectively which show the ghrelin decrease in the elderly and this relationship was statistically significant (P = 0.04). the major source of ghrelin-secreted organ is fundus of stomach and two-third of circulating ghrelin are produced by X/A-like cells of the oxyntic mucosa of the stomach (cells adjunct to capillary network of stomach lamina properia which have
endocrine function), and number of the cells are very low during embryogenesis. With increasing age the number of the cells increase in the first month, and consequently the amount of ghrelin secretion will increase. It is likely that with aging in old age due to the decreased ability of stomach and gastrointestinal tract in secreting ghrelin, the amount of the hormone be changed. These results in the present study with a study done by Makovey and his colleagues in 2007 were comparable (35). Although Dall and his colleagues in 2002, found no significant difference in serum ghrelin levels in different ages, in this study, ghrelin levels in healthy men and men with reduced pituitary activity was measured (36). In a study conducted by Makovey and his colleagues in 2007 a positive correlation between ghrelin and age was found, the study was based on a large population of healthy men and women with a wide age range (35). This correlation was not seen in the present study (P = 0.9), perhaps because the subjects in the present study were close together in age range. We also in the present study investigated the correlation between ghrelin and alkaline phosphatase as indicator of bone turn over. In this study no correlation between ghrelin and alkaline phosphatase was found (P = 0.6). In some studies, no correlation between ghrelin and alkaline phosphatase was found (37, 38), although in another study, ghrelin significantly increased the production of osteocalcin and alkaline phosphatase (22). Several studies have investigated the relationship between ghrelin and BMI. These studies have shown that the ghrelin hormone concentration in obese are less than people with normal body mass index and overweight and in underweight people are less than all subgroups (35, 39, 40-42). However, in studies by Andrea and his colleagues on children with Prader-willi syndrome compared with healthy children (44) and in study by Paik et al. tested Profiles of 24 hours ghrelin in children with Prader-willi syndrome and healthy children (45), and Lindeman and his colleagues studied healthy adults, a significant inverse correlation was found between ghrelin with BMI (46). In addition, in a study by Gonnelli and his colleagues serum ghrelin level in men with osteoporosis was lower than healthy and osteopenia people no statistically significant relationship was found (37). Several studies have been conducted on the correlation between ghrelin and BMD. In a study by Weiss and his colleagues in 2006, no significant correlation between ghrelin and BMD was found in different parts of the body. Pomerants and colleagues in 2007 studied on boys at different stages of maturity and no correlation between ghrelin and BMD was observed (43). In another study on the relationship between ghrelin and other adipocytikines (e.g., leptin, adiponecint) with BMD in adult men, no significant correlation between ghrelin and BMD in lumbar and femoral parts was observed (38).

Gonnelli and his colleagues in 2008 conducted a study on the relationship between ghrelin and adiponektin with bone turnover markers in elderly men and a direct significant relationship between ghrelin and bone density of the femoral neck and total bone density was observed (37). However, in a study by Perez-Castrillón in 2007 on postmenopausal women, the direct relationship between ghrelin and BMD was observed (48).

In most of studies conducted on the total relationship of ghrelin with BMD, no correlation between the ghrelin and BMD was found, although this relationship was reported positively in cell level (21). Another study conducted by Giuseppina on rat osteoblasts primary cell cultures by using RT-PCR showed that ghrelin significantly proliferated osteoblasts (22).

One limitation of the present study was to determine healthy people and their consent to participate in the plan. One reason for the different conclusions in different studies was using different methods to measure the factors and assess BMD. Another reason for the differences in this term is sample size. Ghrelin levels were measured only once in this study, and likely changes in ghrelin levels during the day effect on the regulation of bone density or its changes. Therefore, sampling is recommended more than once daily.

5. CONCLUSIONS

In summary, the findings of this study showed that there was no significant relationship between ghrelin levels and lumbar spine BMD. It is expected that ghrelin could be used as a clinical parameter in the assessment of bone. Choosing a healthy community does not reflect the conclusion on larger population in the same age group. A further study on the relationship between ghrelin and bone density on animal community can pave the path for future studies on this issue. It is seen that ghrelin plays a role in proliferation and differentiation of osteoblasts in cell level, however no relationship was found between that and bone density in many studies which may be due to the fact that bone cells are the source of ghrelin and the produced ghrelin by them have effects on proliferation and differentiation of osteoblasts by paracrine or autocrine and therefore, by measuring the hormone levels in blood, its association with bone mineral density cannot be seen.

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