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ORIGINAL ARTICLE

# Relation Between Secretory Status of Growth Hormone, Serum Concentration of Insulin-like Growth Factor I, and Insulin-like Growth Factor Binding Protein 3 with Bone Mineral Density in Postmenopausal Women

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**Although the decline in sex steroid levels, particularly estradiol, may be largely responsible for age-related bone loss and osteoporotic fractures in older women, the insulin-like growth factor (IGF) system may also play a key role. This study aimed at evaluating the relation between the secretory status of growth hormones (GH), insulin-like growth factor I (IGF-I) and** Insulin-like growth factor binding protein 3 **(IGFBP3) and bone mineral density (BMD) in postmenopausal women.**

**Materials & Methods: In a descriptive cross-sectional study, 150 postmenopausal healthy women were selected from among 1328 patients, referred to Tabriz Sina Hospital for bone densitometry, and divided into three groups according to their bone mineral density (BMD) (normal, osteopenic and osteoporotic). The GH response to provocation by clonidine was assessed in all patients.**

**Results: One hundred and fifty patients with a mean age of 65.6±6.6 years, were enrolled in this study. The impaired GH response to provocation by clonidine was significantly more common in the group with osteoporosis compared to their healthy and osteopenic counterparts (72% vs. 56% and 44%, respectively; p=0.018). Mean levels of serum IGF-I and IGFBP3 were not significantly different in healthy, osteopenic and osteoporotic patients (55.4±20.7 µg/L, 57.5±21.7 µg/L, and 56.7±19.2 µg/L; p=0.880 and 2648.3±786.4 ng/ml, 2374.0±707.2 ng/ml, and 2613.5±1023.6 ng/ml; p=0.217, respectively). There was no strong correlation between the level of serum IGF-I or IGFBP3 and T-Score (r=-0.026, p=0.753 for IGF-1 and r=0.046, p=0.575 for IGFBP3).**

**Conclusion: The results of this study showed that the defective release of GH is more prevalent in postmenopausal women suffering from osteoporosis; such a defect was not observed regarding serums of IGF1 and IGFBP3. Prescription of supplementary doses of synthetic GH might be beneficial in this population.**

**Key Words**: Growth hormone, Insulin-like growth factor I, Insulin-like growth factor binding protein 3, Postmenopausal, Bone mineral density

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## Introduction

Osteoporosis is the most common metabolic bone disease. The national institute of health describes osteoporosis as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fractures.[1] Alteration of many factors that play a role in bone remodeling can contribute to the development of osteoporosis.[2,3]

Bone remodeling is regulated by systemic hormones and locally produced elements acting in unison to maintain bone mass.[4,5] Insulin-like growth factors (IGFs) are synthesized in osteoblasts and are among the most important regulators of bone cell function due to their anabolic effects on the skeleton.[6,7] The key role of the IGF system in the local regulation of bone formation is demonstrated by the finding that approximately 50% of basal bone cell proliferation could be blocked by inhibiting the actions of IGFs, endogenously produced by bone cells in serum-free cultures.[7] However, circulating IGF-I, mainly produced in the liver via regulation by growth hormone (GH) and diet, acts in an endocrine manner as well, which activates bone remodeling and exerts anabolic effects on bone tissues.[8-10] Indeed, there is recent evidence that the GH/IGFI axis plays an important role in maintaining bone mass in adults as well as longitudinal growth of bone in childhood.[11-13]

Approximately 99% of circulating IGFs are bound to six specific high-affinity IGF-binding proteins (IGFBPs) that are produced in osteoblasts and other cell types and modulate IGF action in a positive or negative manner.[14-16] In addition, IGFBPs, per se, may directly affect bone and cartilage metabolism.[17-19] A major portion of IGF-I is bound to IGFBP3, which is a quantitatively predominant IGFBP in the circulation.[20]

Serum IGFBP3 level is considered to be positively regulated by GH and/or IGF-I.[21-23] The serum IGFBP3 level is significantly correlated with BMD, at the mid-radius, when age is taken into account.[24] Furthermore, it has been suggested that serum levels of IGF-I and IGFBP3 would be clinically important predictors of vertebral fracture risk because they were significantly lower in subjects with vertebral fractures than those without fractures during any decade.[24] Thus, it can be speculated that circulating levels of IGF-I and IGFBP3, may affect bone formation and may contribute, at least in part, to osteoporosis.

In postmenopausal women with osteoporosis, administration of growth hormone increases bone turnover.[25] On the other hand, in the skeleton of elderly patients, level of Insulin Like Growth Factor-I (IGF-I) decreases.[26] It may be that age related decreased bone mineral density is a consequence of regional IGF-I deficiency.[27] However, in one comparative study of osteoporotic women and an age matched control group, there were no differences in serum IGF-I and Insulin-Like Growth Factor Binding Protein 3 (IGFBP3) levels.[28] It seems logical that more studies should be done to determine the relation between these factors and osteoporosis. Considering the high prevalence of osteoporosis in the Islamic Republic of Iran,[29,30] and the need to detect relevant risk factors, we conducted this study to determine the relation between postmenopausal osteoporosis and growth hormone secretary status and serum levels of IGF-I and IGFBP3.

## Materials and Methods

In a descriptive-cross sectional study, all patients referred to the Bone Mineral Densitometry (BMD) Center of the Endocrine Department of Sina Medical Center in Tabriz, Iran, over a 12-month period from March 2006 to February 2007, were evaluated. Demographic features, diet, exercise, medical history and clinical status of subjects were evaluated using a questionnaire. Based on the data collected and after considering inclusion and exclusion criteria, finally 150 eligible naturally menopaused patients entered the study. Natural menopause is defined as 12 months of amenorrhea after the final menstrual, period after the age of 45 years. According to the WHO definition,[31] the study population were divided in to three groups, of 50 subjects each; group A. Those with normal BMD (T score >-1) in all measured areas; group B. Those with osteopenia (T score -1 to -2.4) at least in one of the measured areas; group C. Those subjects with osteoporosis (T score <-2.5) at least in

one of measured areas. Subjects were grouped in a manner by which they had no significant differences in diet, exercise, body mass index, medical and drug history. On the basis of demographic parameters, diet, medical history, clinical findings, biochemical studies such as serum calcium, phosphorus, alkaline phosphates, albumin, parathyroid hormone (PTH) and thyroid stimulating hormone (TSH), any patients with possibility of secondary osteoporosis were excluded; patients, who had a positive history of drug use with effects on BMD, GH, IGF-I, or IGFBP3, were also excluded; these medications include corticosteroids, heparin, levothyroxine, lithium, anticonvulsants, long acting GnRH analogues, cyclosporine, chemotherapeutic agents, aluminum containing antacids, (continuous use for over 1 year) and estrogen (as medications affecting BMD); levodopa, clonidine, bromocriptine, propranolol, H2 receptor antagonists, and cholinergic agonists (as GH stimulating agents); somatostatin, phentolamine, yohimbine, phenothiazines, cyproheptadine, methysergide, isoproterenol and glucocorticoids (as GH suppressor agents). Cigarette smokers and alcohol consumers were excluded from the study, as were subjects with cachexia, malnutrition sepsis, renal disorders, liver disorders, and systemic illness and acromegaly. For all the study population, bone mineral densitometry was performed from the lumbar spine and femoral neck regions and, in some subjects the distal radius. Densitometry was performed by DEXA using LUNAR version DPC-MD apparatus. Sampling for measurement of basal serum concentration of GH, IGF-I and IGFBP 3 was done after 10–12 hour overnight fasting. GH stimulation was performed using clonidine 0.4 μg/kg in all eligible subjects. Re-sampling was performed 60 and 90 minutes after clonidine. Serum of all samples were immediately isolated and stored at -25°C. Serum levels of GH, IGF-I, IGFBP3 in fasting samples and GH in post stimulation samples were determined synchronously. GH measured by RIA (Kavoshyar TM, Iran). The inter-intranasal coefficients of variation were 2.8% and 4.2%. respectively. Post stimulation GH increment over 3 to 6 times that at base line (after 60 or 90 minutes of clonidine) was considered normal response and lesser increments were considered abnormal.[32] IGF-I and IGFBP3 were measured by ELIZA (Biosource TM, Belgium). Intra- and interassay coefficient of variations of the measurement were 5.5-10.1% and 6.2-9.7%, respectively. All laboratory studies were performed at the Plasma Medical Laboratory, Tabriz, Iran).

Statistical Analyses were done using SPSS TM version 15. Data obtained are presented as mean±SD, frequency and perc-entage where needed. Quantitative variables were analyzed by student – T or ANOVA tests. Qualitative, categorical variables were analyzed by contingency tables and Chi square or Fishers exact test, as required. For detection of correlation Spearman coefficients were used. Results were considered significant when P Value was $\leq 0.05$.

## Results

The mean age of patients was 56.6±6.6 years, range 46-77 years, with no significant differences between groups regarding their mean age (p=0.09). Table 1 shows the variables studied in the three groups. Mean T-score was -1.72±1.38 (Range -5.9 to 2.4). Mean serum level of GH was 1.45±1.25 ng/mL. The latter value was 1.67±1.37 ng/mL in group A, 0.88±0.59 ng/mL in group B and 1.83±1.39 ng/mL in group C. The mean poststimulation serum GH level at 60 minutes was 2.76±1.68; it was 3.01±1.6 in group A, 2.04 ± 1.21 in group B and, 3.24±1.93 in group C. Mean serum GH levels at 90 minutes after clonidine administration, were 3.15±1.69, 3.49±1.66, 2.55±1.71 and 3.42±1.63, in groups A, B, and C respectively. Normal responses of GH increment after clonidine stimulation were seen in 64 (42.7%) subjects. Abnormal response was reported in 86 (57.3%) of participants. Normal and abnormal responses

seen were in 22 and 28 in group A respectively; in group B these were 28 and 22, and 12 and 38 in group C, being significantly high in group C respectively (p=0.018).

**Table 1. Variables studied in the three groups**

| Variables | Normal BMD (Group A) | Osteopenia (Group B) | Osteoporosis (Group C) | P |
|---|---|---|---|---|
| Number | 50 | 50 | 50 | NS |
| Age (yr) | 55.4±6.4 | 56.1±6.0 | 58.2±7.0 | NS |
| BMI (kg/m2) | 28.1±3.2 | 29±2.2 | 27.6±2.6 | NS |
| Duration of Menopause (yr) | 5.6±1.8 | 6.1±2.7 | 6.8±3.1 | NS |
| Physical activity | | | | |
| Low* | 52% | 58% | 70% | 0,05¥ |
| Moderate** | 46% | 42% | 30% | NS |
| Severe*** | 2% | 0 | 0 | NS |
| T-Score (mean±SD) | -0.2±0.57 | -1.72±0.42 | -1.72±1.38 | <0.001 ¶ |
| Basal GH (ng/mL) | 1.67±1.37 | 0.85±0.59 | 1.83±1.39 | § <0.001 |
| GH at min 60 (ng/mL) | 3.01±1.60 | 2.04±1.21 | 3.24±1.93 | ‡ <0.001 |
| GH at min 90 (ng/mL) | 3.49±1.66 | 2.55±1.71 | 3.42±1.63 | † <0.007 |
| GH Response to Clonidine | | | | |
| Normal# | 22(44%) | 28(56%) | 12(24%) | 0.018¢ |
| Abnormal# | 28(56%) | 22(44%) | 38(76%) | |
| Serum IGF-1(µg/L) | 55.4±20.7 | 57.5±21.7 | 56.7±19.2 | 0.880 |
| Serum IGFBP3 (ng/mL) | 2648±786 | 2374±707 | 2613±1023 | 0.217 |

NS=Nonsignificant; ¥ Comparison of groups A and C; ¶ Comparison of groups A and C; §Comparison of groups B and C; ‡Comparison of groups A and C; †Comparison of groups A and C; ¢ Comparison normal and abnormal responses in group A. * Lesser than 1 hour walking in a week. ** At least 1 hour walking in a week. *** Regular daily exercise. # Post stimulation GH increment greater then 3 to 6 times of base line after 60 or 90 minute of clonidine was considered normal response and lesser increments considered abnormal

Mean serum level of IGF-I was 56.58±20.52 in the whole study population, minimum and maximum values being 22 and 120 mg/L respectively; mean serum levels of IGF-I in separate groups are shown in Fig. 1. There were no significant differences in these three groups (p=0.880).

Mean serum IGFBP3 level was 2545.29± 852.90 ng/mL (range 251-7398 ng/ml); these levels in different study popula-tions are shown in Fig. 1; there were no statistically significant differences between the groups (p=0.217).

Mean T - score in subjects with normal response to clonidine was -1.54±1.21 and in abnormal responders was -1.85±1.48. There was no significant difference between the two groups (95% CI:–0.75–0.15, p=0.184).

A comparison of serum IGF-I levels between groups A and B, showed no signif-icant differences (95% CI:-10.53–6.37, p=0.626); neither was mean serum IGFBP3 significantly different in these groups (95% CI:-22.53-571.13, p=0.070). The rates of abnormal response to clonidine were not statistically different in these groups either (OR=1.62; 95% CI: -3.22-1.78, p =0.230).

(95% CI: -9.18–6.74, p=0.76); neither were means for IGFBP3 significantly different (95% CI:-327.49–397.05, p=0.626). The number of subjects with abnormal responses to clonidine in these two groups also were not statistically different (OR= 0.49; 95% CI: 0.22-1.14, p = 0.096).

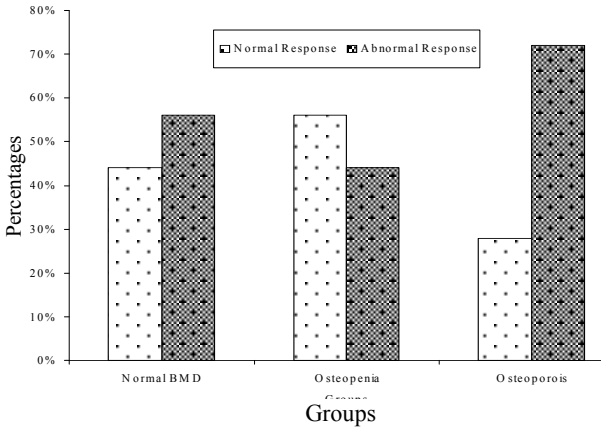


**Fig. 1. Percentages of normal and abnormal responders to stimulation by clonidine in groups studied**

Comparing group A and C, means of serum IGF-I level were not significantly different

Comparison of groups B and C, showed that the means of serum IGF-I level in these two group were not significantly different (95% CI: -7.30-9.02, p=0.835), as was the case for means of serum IGFBP3 (95% CI: -588.70–109.66, p=0.77). The number of subjects with abnormal responses to clonid-ine in these two groups were signific-antly greater in group C in comparison with group B (OR=0.31; 95% CI: 0.13-0.70, p=0.005). Table 2 shows correlation of different variables in the three study groups.

**Table 2. Correlation of different variables in study groups**

| | Normal BMD (Group A) | | Osteopenia (Group B) | | Osteoporosis (Group C) | |
|---|---|---|---|---|---|---|
| **Variable** | **r** | **P** | **r** | **P** | **r** | **P** |
| Age and T-Score | o.384 | 0.006 | 0.125 | 0.388 | 0.081 | 0.574 |
| IGF-1 and T-Score | -0.086 | 0.553 | -.008 | 0.956 | 0.052 | 0.721 |
| IGFBP-3 and T-Score | 0.057 | 0.696 | -0.028 | 0.845 | 0.121 | 0.403 |
| Age and IGF-1 | 0.040 | 0.780 | 0.254 | 0.076 | 0.264 | 0.064 |
| Age and IGFBP-3 | 0.06 | 0.680 | -0.193 | 0.179 | -0.162 | 0.261 |

## Discussion

It is known that the GH secretion rate as well as serum levels of IGF-I and IGFBP3 decrease with age.[12] It is also known that GH is one of the major regulators of circulating levels of IGF-I and IGFBP3[15] and that a deficiency in GH is associated with a severe reduction in serum levels of these stimulatory IGF system components. In the present study, we found that abnormal response of GH to a provocative test was significantly more common in osteoporotics, in comparison with osteopenic or subjects without abnormal bone density. Dequeker et al, showed that secretion of GH after administration of L-arginine is significantly lower in osteoporotic patients in comparison with a control group.[33] It seems that the age dependent decline of GH secretory capacity differs in postmeno-pausal women. Factors such as dietary status, level of physical activity, genetic differences, or other unknown parameters can explain this inhomogeneity of GH secretory pattern in postmenopausal women. Although no signif-icant variation in physical activities between study groups was seen, osteopor-otics were found to have the lowest levels of physical activity.

Sugimoto et al, studied the effects of low dose recombinant GH in elderly women with osteoporosis; they showed that administration of GH increased BMD and improved bone status in the study population.[34] This can be considered as an indirect evidence of the GH

role in bone mineral density status. Multiple studies have shown that adult growth hormone deficiency can reduce BMD, while GH administration can reverse this process;[35-41] these findings also are some what similar to those of our study.

Study groups were also compared with each other. Findings showed that the rate of abnormal responses to clonidine were significantly higher only in osteoporotic patients in comparison with osteopenic subjects (OR=0.31; 95% CI: 0.13–0.70, p=0.005). However there were no such differences between osteoporotic and normal BMD groups. Probably the changes in the secretory pattern of GH play an important role in progression of osteopenia to osteoporosis in postmenopausal years while the effect on normal bone is negligible. Results of some experiments support this conclusion.[42,43] However, with the paucity of similar studies, further investigations can be helpful in precise deductions.

In this study we compared serum levels of IGF-I and IGFBP3 in subjects with normal BMD, osteopenia and osteoporosis, and in the three study groups, found no significant differences between these variables (p=0.880, 0.217 respectively).

Results of studies in this field are very inconsistent. Kassem et al, in a study on postmenopausal women with osteoporosis of the lumbar spine showed that there were no significant differences in serum levels of IGF-I and IGFBP3 between case and control groups.[28] Bennett et al, studied 57 women with normal BMD and 29 osteoporotic postmenopausal women, and found no statistically significant difference in serum IGF-I levels.[44]

On the other hand, some studies have shown that serum level of IGF-I and IGFBP3 are significantly lower in osteoporotic pati-ents compared with controls.[45-51] In our study there was no significant correlation between serum IGF-I level and T-score serum level of IGFBP3 and T-score. Yamaguchi et al, studied 193 Japanese postmenopausal women, and found no significant correlation between serum IGF-I level and BMD of femoral neck; neither did they report any meaningful correlation between serum IGFBP3 level and BMD of most areas of the skeleton except for distal radius.[52]

The end point of treatment for osteoporosis is the prevention of bone fractures attributed to it. Therefore, it is of great importance to predict the risk of osteoporotic fractures. From this point of view, many investigators have tried to establish the threshold of osteoporotic fractures of the hip and spine.[53-56] However, it became evident that there is no absolute threshold of BMD for predicting bone fractures that shows sufficiently high sensitivity and specificity. Measurements of biochemical bone markers such as osteocalcin, pyridinoline, and deoxypyridinoline are helpful in predicting the rate of bone loss; however it is still difficult to predict the risk of osteoporotic fractures from these measurements.[57,58] Accordingly, some studies showed that serum levels of IGF-I and IGFBP3 were apparently lower in subjects with vertebral fractures than those without fractures and that their serum levels were strongly associated with the presence of vertebral fractures in postmenopausal women.[52] Of these Yamaguchi and his colleagues concluded that IGF-I and IGFBP3 in the circulation might play some important role in maintaining bone mass not only quantitatively but also qualitatively; they suggest that serum levels of IGF-I and IGFBP3 could be surrogate markers for evaluating the severity of osteoporosis by efficiently predicting the risk of vertebral fractures.[52] In our study, the relation between fracture rate and serum levels of GH and their related compounds was not evaluated. This subject can be a topic for future studies to explain the lack of relation between serum levels of IGF-I and IGFBP3 and status of bone mineral density in our findings. Probably qualitative features of bone have greater relation to the GH system than quantitative features.

In the Karrasik survey of 101 female patients no significant correlation between

serum IGF-I level and BMD of lumbar spine was seen;[59] the Martini et al study showed no significant correlation between IFG-I and IGFBP3 serum levels and BMD.[60] However, Amin et al, showed that, in postmenopausal women with reduction of IGF-I and IGFBP3 serum levels BMD decreases significantly.[61] Gillberg reported a similar finding in Swedish men.[62] Kim et al, in study of 65 postmenopausal women showed that there is a significant correlation between IGF-I and IGFBP3 serum levels and BMD.[37]

As mentioned, the results of various studies in this field are divergent. One reason for the inhomogeneity of findings is the involvement of multiple factors in the pathogenesis of osteopenia and osteoporosis as a whole and in postmenopausal women specifically. In our study, there were no significant correlations between serum IGF-I and IGFBP3 levels and age of studied patients (r=0.13, p=0.111; r= 0.158, p=0.054 respectively), similar to the Karasik study results.[59] Martini also showed there is no significant correlation between serum levels of IGFBP3 and age in postmenopausal women.[60] However, in other studies the presence of such a correlation is reported; with increasing of age, serum levels of IGF-I and IGFBP3 decrease significantly.[52,61,64] Our study population of three groups were age matched, which as a possible counfounding variable would be hence eliminated.

In the present study, 150 subjects were selected from a 1328 primary study population. Diet, exercise, cigarette smoking, medications with effects on bone, and backgr-ound diseases (such as malignancies, metabolic bone diseases, and renal disorders) were factors that were evaluated in selected subjects, excluding patients with these criteria. In fact, this was the main superiority of our study design; in other studies this problem is either considered slightly or not at all.[52] The other reason for inhomogeneity of findings in various studies is the different methods of evaluation of study parameters, and different procedures of densitometry. Variations of sample size can also explain the diversity in findings.

Some studies have showed that BMD in a special area of the skeleton cannot reflect total body BMD;[65,66] considering this, in the future studies, application of total body BMD can be helpful in obtaining precise results. On the other hand, it has been shown that serum IGF-I levels are not a good predictor of bone content of this factor.[67-69] hence considering these facts, we need further studies with better control of confounding factors in this field.

A major limitation of our study was the smal sample size. As lack of significant diff-erences between groups can be explained by small sample sizes, for study of relationship of IGF-I and IGFBP3 with BMD we recom-mend controlled studies with larger sample size. Also direct measurement of bone IGF-I will help to obtain more precise conclusions.

In conclusion, in postmenopausal women, abnormal responses of growth hormone after stimulation with clonidine were seen more frequently in osteoporotics compared to oste-openics or those with normal BMD. Mean serum IGF-I and IGFBP3 levels in postmen-opausal woman with normal BMD, osteo-penia or osteoporosis were not signific-antly different. There was no significant correlation between serum IGF-I and IGFB-P3 level and BMD. Therefore administration of synthetic GH for therapeutic purposes can be considered in osteoporotic postmenopa-usal women after further studies. A qualitat-ive bone study would help facilitate studies of the relation between serum GH level and related compounds. We recommend applica-tion of total body BMD in future studies.

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