Serum IGF-1 and IGFBP-3 Levels in Middle Aged Turkish Males: Relationships with Bone Mineral Density and Markers of Bone Turnover (Male Osteoporosis & IGF-1, IGFBP-3)

Orta Yaşlı Türk Erkeklerinde Serum IGF-1 ve IGFBP-3 Seviyeleri: Kemik Mineral Yoğunluğu ve Kemik Yapım-Yıkım Belirteçleri ile İlişkileri (Erkek Osteoporuzu & IGF-1, IGFBP-3)

Melek Sezgin, Burak Çimen*, Arzu Kanık**, İsmet As, Neslihan Erçetin*, Nurgül Arıncı İncel, Günşah Şahin

*Department of Physical Medicine and Rehabilitation, *Department of Biochemistry and Department of Biostatistics, Medical Faculty of Mersin University, Mersin, Turkey*

Summary

Aim: The aim of this study was to determine whether circulating levels of insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) were associated with bone mineral density (BMD) and bone turnover markers in middle aged Turkish males.
Patients and Methods: At the beginning, a total of 160 Turkish men aged between 35 and 65 years were included to this study. The final sample comprised of 112 men because 48 men were excluded from the study. BMD of the spine and the hip was measured with dual energy x-ray absorptiometry. After an overnight fasting, serum IGF-1, IGFBP-3, intact parathyroid hormone, 25-hydroxy vitamin D, osteocalcin, C-terminal telopeptide, calcium, phosphorous and alkaline phosphatase levels were measured. Urinary concentrations of calcium, phosphorous and creatinine were also estimated.
Results: Twenty-one men (18.8%) had a bone mineral density of ≤ -2.5 SD (T score). There was a significant difference in IGF-1 levels between men with normal BMD and men with reduced BMD (132.5 ± 38.1 and 116.1 ± 40.6 respectively and p: 0.04). Serum IGF-1 levels were positively correlated with BMD of the lumbar spine (r: 0.28, p:0.006), but there was no correlation between IGFBP-3 and BMD of any sites tested. IGF-1, IGFBP-3 and BMD were not correlated with bone turnover markers except serum alkaline phosphatase level.
Conclusion: Serum IGF-1 levels were lower in men with reduced BMD and positively correlated with BMD of the lumbar spine. Neither IGF-1 nor IGFBP-3 was correlated with bone turnover markers. Further studies of these factors in skeletal cells are needed to explain their role in the pathophysiology of idiopathic male osteoporosis.
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Key words: Idiopathic male osteoporosis, insulin-like growth factor-1, insulin-like growth factor binding protein-3, and bone turnover markers

Ozet

Amaç: Bu çalışmanın amacı orta yaşlı Türk erkeklerinde serum insulin benzeri büyüme faktörü-1 (IGF-1) ve insulin benzeri büyüme faktörü bağlayıcı protein-3 (IGFBP-3)'ün kemik mineral yoğunluğu ve kemik yapım yıkım belirteçleri ile ilişkili olup olmadığını göstermekti.
Hastalar ve Yöntem: Başlangıçta bu çalışmaya 35 ile 65 yaşları arasında 160 Türk erkeği dahil edildi. Daha sonra 48 hasta çalışma dışı bırakıldığı için son örneklem 112 erkeği içerdi. Omurga ve kalçanın kemik mineral yoğunluğu (KMY) dual enerji x-ray absorptiometri ile ölçüldü. Bir gecelik açlık sonrası, serum IGF-1, IGFBP-3, intakt parathormon, 25-OH vitamin D, osteokalsin, C-terminal telopeptid, kalsiyum, fosfor, ve alkalen fosfataz seviyeleri ölçüldü. Kalsiyum, fosfor ve kreatininnin idrar konsantrasyonları da tesbit edildi.
Bulgular: Yirmi bir erkekde (% 18.8) kemik mineral yoğunluğu T skoru -2.5 SD'a eşit veya altında idi. Normal ve azalmış KMY'si olan hastalar arasında IGF-1 düzeylerinde anlamlı farklılık vardı (sırasıyla 132.5 ± 38.1 ve 116.1 ± 40.6, p: 0.04). Serum IGF-1 düzeyleri lomber omurganın KMY'si ile pozitif korele idi. Fakat, IGFBP-3 ve test edilen yerlerin hiçbirinin KMY'si arasında korelasyon yoktu. IGF-1, IGFBP-3 ve KMY serum alkalen fosfatazı hariç kemik yapım yıkım belirteçleri korele değildi.
Sonuçlar: Serum IGF-1 seviyeleri KMY'si azalmış olan erkelerde daha düşüktü ve lomber omurganın KMY'si ile pozitif olarak korele idi. Ne IGF-1 ne de IGFBP-3 kemik yapım yıkım belirteçleri ile korele değildi. İskelet hücrelerindeki bu faktörlerin, idiopatik erkek osteoporozunun patofizyolojisindeki rolünü açıklamak için ek çalışmalara gereksinim vardır.
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Address for Correspondence: Dr. Melek Sezgin, Liparis Plaza-1, H Blok, 7/15 Mezitli, Mersin, Turkey
Phone: +90 324 337 43 00/1140 Fax: +90 324 337 43 05 E-mail: msezgin@mersin.edu.tr

Introduction

Osteoporosis is one of the commonest metabolic bone diseases, and its prevalence is expected to rise as the population grows older. Although osteoporosis is less prevelant in men than in women, the morbidity and mortality due to male osteoporosis is higher (1,2). The major causes of osteoporosis in men are hypogonadism, hypercortisolism and excessive alcohol intake. Other abnormalities such as hyperthyroidism and malignancy are less commonly responsible for osteoporosis. All above mentioned disorders account for osteoporosis in approximately 60% of men (3,4). The remaining subset of men with idiopathic osteoporosis has been poorly characterized. With no readily identifiable causes of reduced bone density in men with idiopathic osteoporosis, it is attractive to consider the possibility that this disorder is the result of fundamental abnormalities of bone cell function or of the hormonal or paracrine pathways that regulate the bone cell metabolism (5,6).

Growth hormone (GH) and its major mediator insulin like growth factor 1 (IGF-1) are thought to be important both in attainning peak bone mass and maintaining adult bone mass (7,8). The synthesis of circulating IGF-1 occurs principally in the liver; osteoblasts also contribute to IGF-1 production (9). Invitro and invivo studies have shown IGF-1 to enhance type 1 collagen production and bone matrix apposition rates and decrease degradation of bone collagen (10,11). A major portion of IGF-1 is bound to IGFBP-3 which is a quantitatively predominant IGFBP in the circulation. Serum IGFBP-3 level is thought to be positively regulated by GH and / or IGF-1 (12,13). It has been suggested that IGFBP-3 might augment the effect of IGF-1 on the bone (14).

Recent studies have focused on the role of GH and IGF-1 in the regulation of bone metabolism in men. In elderly men, bone mineral density (BMD) is not correlated with IGF-1 concentrations. In contrast, studies including younger men suggested a weak possitive correlation between serum IGF-1 and BMD at some skeletal sites (15-20). However, these studies are not easily comparable because they were performed in mixed cohorts composed of men and women or in small samples of different age groups and based on different statistical approaches.

There have also been reports indicating an association between low IGFBP-3 and BMD in both men and postmenopausal women. Others have shown only a weak association between IGFBP-3 and BMD in men and no association in women (18, 21-23).

Therefore, we attempted to evaluate the effects of serum IGF-1 and IGFBP-3 levels on BMD at various skeletal sites in Turkish adult men. In addition, we investigated the relationship between IGF-1, IGFBP-3 and bone turnover markers.

Material and Methods

Study Population

This study included a total of 160 Turkish adult men referred to the outpatient clinic of Physical Therapy and Rehabilitation Department at Mersin University Hospital. Each subject was given a questionnaire composed of questions on smoking habits, alcohol consumption, exercise habits, history of chronic diseases, previous and present medication, previous fractures, back pain and family history of fracture. Dietary calcium intake was evaluated with a simplified food frequency questionnaire. History of GH deficiency or GH excess, hypogonadism and treatment with androgens or a disease or medication known to influence bone and calcium metabolism were considered as exclusion criteria. At the beginning of the study, thirty-eight men were excluded because they had diabetes mellitus, cronic obstructive lung disease, ankylosing spondylitis, stroke, myastenia gravis, cronic hepatic disease, osteomalasia, hyperparatyroidism, Parkinson's disease and treatments of antiepileptics, diuretics or steroids etc. Later, 10 more men were excluded from the study because their biochemical analyses or measurements of bone mineral density were missing. The final sample comprised of 112 men aged between 35 and 65 years. Weight was measured with a beam balance and height with a stadiometer in all subjects. Body mass index (BMI) was calculated as in the following: weight (kilograms) / height (meters). All subjects gave informed consent to participate in the study.

BMD and Biochemical Measurements

BMD was measured at the lumbar vertebrae (L2-4) and hip (femoral neck, Ward's triangle and trochanter) with dual energy x-ray absorptiometry and a Norland XR-46 scanner (Ford Atkinson, WI. USA). The coeffient of long-term variations of BMD measurements with the scanner is 2 % at the lumbar spine and 2.4% at the neck of the femur.

After an overnight fast, blood samples were taken at 8:00 am and a 24-hour urine collection completed. Serum IGF-1, IGFBP-3, calcium, phosphorous, alkaline phosphatase (ALP), intact parathyroid hormone (PTH), 25-hydroxy vitamin D (25OH D), osteocalcin (OC) and C-terminal telopeptide (CTx), were measured in all subjects. Urinary concentrations of calcium, phosphorous and creatinine were also estimated. Specimens collected to determine IGF-1 and IGFBP-3 were centrifuged immeditely after they were obtained and their sera were extracted. The sera were kept at -20° for one month when IGF-1 and IGFBP-3 were determined. Other measurements were made immediately after specimens were taken.

IGF-1 (normal range: 55-358 ng/mL) and IGFBP-3 (normal range: 2.4-9.5 µg/mL) levels were measured with the Immulite 1000 automated analyzer (Bio-DPC) and

solid phase enzyme labelled chemiluminescent immunometric assay. For IGF-1 and IGFBP-3 the interassay and intraassay coefficients of variation were 6.6%-8.5% and 3.5%-4.2% respectively.

Both intact OC (1-49 amino acids) and the large N-terminal fragment (1-43 amino acids) occur in blood. Intact OC is unstable due to protease cleavage between amino acids 43 and 44. The N-terminal region resulting from the cleavage is considerably more stable. The assay detects the N-terminal fragment. OC (normal range: 14-42 ng/mL) levels were determined with electrochemiluminescence immunoassay (Roche Diagnostic Modular Analytics E170). Especially relevant fragments of collagen type-I are the C-terminal telopeptides. In the C-terminal telopeptides, α-aspartic acid is converted to the β-form of aspartic acid as the bone ages (β-CTx). These isomerised telopeptides are specific for the degradation of type-I collagen dominant in the bone. The assay specifity is guaranteed through the use of two monoclonal antibodies each recognizing linear β-8AA octapeptides (EKAHD- β-GGR). CTx levels (normal range: <0.584mL) were determined with electrochemiluminescence immunoassay (Roche Diagnostic Modular Analytics E170). Serum intact PTH levels were determined by electrochemiluminescence immunoassay (Roche Diagnostic Modular Analytics E170). 25OH D levels were detected by using chromatographic method (HPLC Agilent 1100). Urinary and serum calcium levels were determined with a o-cresolphtalein method, phosphorous concentrations with phosphomolibdat, creatinine concentrations with enzymatic colorimetric method and ALP enzyme activities with p-nitrophenylphosphate (Roche Cobas Integra 800).

Statistical Analyses

Descriptive characteristics of the study population were expressed in numbers and percentages (Table 1). Mean and standard deviations of baseline measurements were used to determine. These statistics are given in Table 2 and 3. Multivariate analyses were used to explain the relation between dependent variables (serum IGF-1, IGFBP-3 and BMD) and independent variables (age, BMI, daily calcium intake, smoking, alcohol intake, chronic diseases, medications, history of fracture, back pain and exercise habits). Partial correlation analyses were made to adjust for age and BMI and Pearson correlation analysis to determine the relationship between IGF-1, IGFBP-3, BMD and bone turnover markers. The results of these analyses are given in Tables 4. In addition, the subjects were assigned into two new groups: those with 'low BMI' and those with 'normal BMI'. Independent samples t test was used to compare these groups in terms of serum IGF-1 and IGFBP-3 (Figures 1-2). The scatter plot and regression fit line plotted were used to show the relationship between age and serum IGF-1 and IGFBP-3 in each subgroup (Figures 3-4). All statistical analysis were performed with SPSS software, version 12. P< 0.05 was considered significant.

Results

The mean age of 112 subjects included in the study was 51.9 years and their mean BMI was 25.9kg/m^2. Sixty-one patients smoked more than 5 cigarettes and twelve patients consumed fewer than 2 units of alcohol (i.e. <18g) a day. Forty-eight patients did exercise for 30-60min at least three times a week. The mean daily calcium intake was 314mg. Seven patients (6.3%) had a history of fracture, fourteen patients (12.5%) had a family history of fracture and 47 patients (42%) had backpain. Besides, 44 patients (38.7%) had a chronic

Table 1. Descriptive variables of the study population

Variables	N	%
Education		
Primary school	14	12.5
High school	40	35.7
University	58	51.8
Cronic Disease		
No chronic diseases	69	61.3
Hypertension (HT)	19	17.1
Coronary artery disease (CAD)	12	10.8
HT+CAD	4	3.6
Bening Prostate hypertrophy	8	7.2
Medication		
No medication	69	61.6
1 or 2 drugs	28	25.0
Three or more drugs	15	13.4
Smoking habits		
Non-smoker	51	45.5
Ex-smoker	27	24.1
Smoker	34	30.4
Consumption of alcohol		
No consumtion	100	89.3
Consuming or has given up alcohol	12	10.7
Regular Exercise		
Present	48	43.2
Absent	64	56.8
Previous Fracture		
Present	7	6.3
Absent	105	93.8
Family History of Fracture		
Present	14	12.5
Absent	98	87.5
Back Pain		
Present	47	42.0
Absent	65	58.0

disease (hypertension, coronary artery disease, benign prostate hypertrophy) and medication.

Descriptive and baseline characteristics of the subjects are shown in Tables 1 and 2. The mean IGF-1 level was 121.5 ± 40.40 ng/mL and the mean IGFBP-3 level was 4.40 ± 1.20 ng/mL. The mean BMD was 0.96 ± 0.16 g/cm^2 in the lumbar spine, 0.85 ± 0.14 g/cm^2 in the femoral neck, 0.72 ± 0.11 g/cm^2 in the trochanter and 0.63 ± 0.15 g/cm^2 in the Ward's triangle. The mean T scores at the lumbar spine and femoral neck were -1.28 ± 1.33 and -0.76 ± 1.02 respectively (Table 3). Twenty-one subjects (18.8%) were found to have BMD T scores of ≤ -2.5 SD in at least one skeletal site tested. Fifty-four subjects (48.2%) were found to have BMD T scores of < -1SD or > -2.5 SD, while the rest had bone density T scores of ≥-1 SD.

Table 2. Baseline characteristics of the study population

	mean±sd	min-max
Age (year)	51.9 ±7.6	35-65
Height (cm)	172.4 ± 5.9	162-187
Weight (kg)	77.3 ± 11.2	52-115
BMI (kg/m^2)	25.9 ± 3.4	18.4-34.6
Serum Ca (mg/dL)	9.7 ± 0.5	8.4-11.6
Serum P (mg/dL)	3.4 ± 0.5	2.2-5.0
ALP (U/L)	129.7 ± 62.0	36.2-270.0
iPTH (pg/mL)	41.2 ± 12.9	5.45-85.3
25OH D (µg/L)	43.8 ± 20.5	20.0-120.0
OC (ng/mL)	24.5 ± 7.9	9.9- 52.1
CTx (ng/mL)	0.41 ± 0.22	0.09-1.19
Urine Ca (mg/day)	212.79 ± 130.28	14.34- 826.0
Urine P (g/day)	0.65 ± 0.26	0.04-1.68
Urine Cr (mg/day)	1291.3 ± 440.1	104.4-2944.0
ILGF-1 (ng/mL)	121.5 ± 40.40	2.30- 250.00
ILGFBP-3 (µg/mL)	4.40 ± 1.20	2.00-9.10

BMI- Body mass index, Serum Ca- serum calcium, Serum P- serum phosphorous, ALP- alkaline phosphatase, iPTH- intact parathyroid hormone, 25OHD- 25-hydroxy vitamin D, OC- osteocalcin, CTx- C-terminal telopeptide, Urine Ca- urine calcium, Urine P- urine phosphorous, Urine Cr- urine creatinine, IGF-1- insulin-like growth factor-1, IGFBP-3- insulin-like growth factor binding protein-3

Although there was a significant difference in serum IGF-1 levels between men with normal BMD and men with reduced BMD (132.5 ± 38.19 ng/mL and 116.1 ± 40.61 ng/mL respectively and p: 0.04) (Figure 1), there was no difference in serum IGFBP-3 levels (4.42 ± 1.22 ng/mL, 4.39 ± 1.20 ng/mL and p: 0.9) (Figure 2). Multivariance analyses were made to determine effects of age, BMI, daily calcium intake, smoking, alcohol consumption, chronic diseases, medication, history of fractures, back pain and exercise habits on serum IGF-1, IGFBP-3 and BMD. Back pain, history of fractures and alcohol consumption had a significant effect on IGFBP-3 (p:0.02, p.0.02 and p:0.03 respectively). Using medication had an effect on Ward's BMD (p:0.04), doing exercise had an effect on femoral neck BMD (p:0.04) and age had an effect on femoral neck (p:0.005) and trochanter (p:0.04) BMD. After age and (51.7 years) and BMI (25.9 kg/m^2) were adjusted, the relation between serum IGF-1 and IGFBP-3 and BMD and bone turnover markers was investigated (Table 4). There was a positive correlation between serum IGF-1 levels and BMD in the lumbar spine (r: 0.28, p:0.006). Whereas circulating IGFBP-3 levels were positively corretated with serum IGF-1 levels (r:0.45, p:0.0001), no correlation was found between IGFBP-3 and BMD in any sites tested. There was no correlation between IGF-1, IGFBP-3 and bone turnover markers. There was a positive correlation between BMD at the lumbar spine and serum alkaline phosphatase (r: 0.19, p:0.03). But, no correlation was found between BMD and other bone markers.

Discussion

This study revealed that 18.8 % of Turkish adult men had a bone density with T scores of ≤ -2.5 SD. The definition of low BMD used in this study was based on the WHO diagnostic criteria (24,25). This study showed that serum IGF-1 levels were lower in individuals with decre-

Table 3. T scores and bone mineral densities of the study population

	mean±sd	minimum	maximum
LSTS	-1.28 ± 1.33	-3.80	2.02
FNTS	-0.76 ± 1.02	-2.70	2.17
LSBMD values (g/cm^2)	0.96 ± 0.16	0.66	1.36
FNBMD values (g/cm^2)	0.85 ± 0.14	0.36	1.24
TBMD values (g/cm^2)	0.72 ± 0.11	0.48	0.99
WBMD values (g/cm^2)	0.63 ± 0.15	0.39	1.14

LSTS- lumbar spine T score, FNTS- femoral neck T score, LSBMD- lumbar spine bone mineral density, FNBMD- femoral neck bone mineral density, TBMD- trochanter bone mineral density, WBMD- Ward's triangle bone mineral density

ased BMD compared to men with normal BMD. There was a significant positive correlation between IGF-1 and lumbar spine BMD. Although there was a positive correlation between serum levels of IGFBP-3 and IGF-1, no correlation was found between IGFBP-3 and BMD at the sites tested. In addition, IGF-1, IGFBP-3 and BMD were not correlated with bone turnover markers except serum ALP level.

Earlier studies have shown reduced serum levels of IGF-1 in men with idiopathic osteoporosis (IO) (6,26,27). In addition, Ljunghall and Reed et al. demonstrated a significant correlation between IGF-1 levels and spine and forearm bone density and osteoblastic surface (26,27). Similarly, Kurland et al. found low IGF-1 levels in men with IO, which was correlated with lumbar spine bone density. They claimed that low IGF-1 levels may reflect the reduction in bone formation, which could be demonstrated by histomorphometry (6). Thereafter, Janssen et al. measured serum IGF-1 levels in 103 healthy males. IGF-1 was correlated with BMD at the lumbar spine (28). Gillberg et al. showed that serum IGF-1 and IGFBP-3 levels were positively correlated with BMD of the whole body, distal and ultradistal radius and femoral neck in 55 Swedish men (19).

Table 4. Partial correlation between circulating IGF-1 and IGFBP-3 levels with bone turnover markers and BMD

	IGF-1 r	IGF-1 p	IGFBP-3 r	IGFBP-3 p
Serum Ca	0.06	0.5	0.07	0.4
Serum P	0.01	0.8	-0.01	0.9
ALP	0.11	0.2	-0.05	0.6
i PTH	-0.06	0.5	0.13	0.2
25OH D	-0.06	0.5	0.03	0.7
OC	0.009	0.9	-0.09	0.3
CTx	0.01	0.8	-0.01	0.8
Urine Ca	-0.03	0.7	-0.06	0.5
Urine P	-0.13	0.2	0.04	0.7
Urine Cr	-0.13	0.2	-0.02	0.7
LSBMD	0.28	0.006*	0.06	0.5
FNBMD	0.11	0.2	-0.16	0.11
TBMD	0.14	0.18	-0.07	0.4
WBMD	0.12	0.2	-0.06	0.5

IGF-1- İnsulin-like growth factor-1, IGFBP-3- insulin-like growth factor binding protein-3, Serum Ca- serum calcium, Serum P- serum phosphorous, ALP- alkaline phosphatase, iPTH- intact parathyroid hormone, 25OHD- 25-hydroxy vitamin D, OC- osteocalcin, CTx- C-terminal telopeptide, Urine Ca- urine calcium, Urine P- urine phosphorous, Urine Cr- urine creatinine, LSBMD- lumbar spine bone mineral density, FNBMD- femoral neck bone mineral density, TBMD- trochanter bone mineral density, WBMD- Ward's triangle bone mineral density

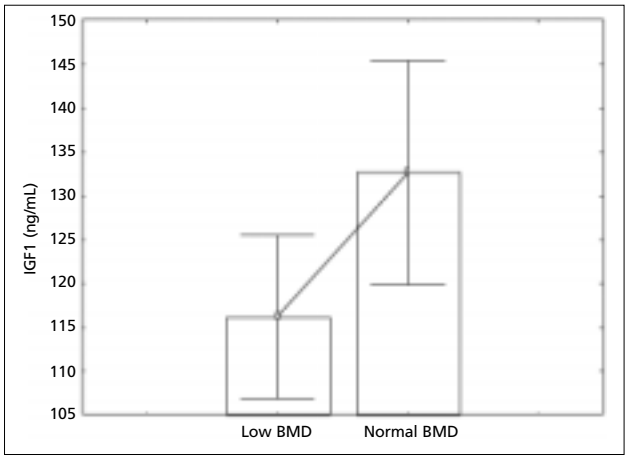

Figure 1. The Comparison of groups in terms of serum IGF-1

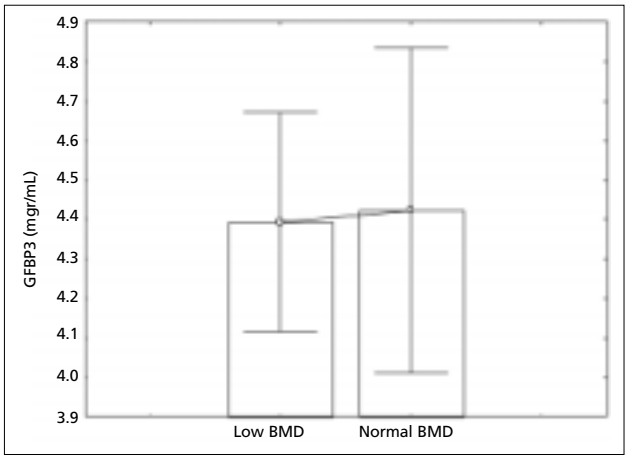

Figure 2. The Comparison of groups in terms of serum IGFBP-3

In contrast, Krassas et al. in their study on in 363 healthy Greek men reported serum GH, IGF-1 and IGFBP-3 levels did not differ in individuals with decreased BMD compared to men with normal bone density. However, they showed a positive correlation between IGF-1 and IGFBP-3 levels and BMD in 2 skeletal sites (LS, FN) (29). Gürlek et al. showed that serum IGF-1 levels was not correlated with BMD and bone turnover markers in 14 Turkish men aged over 60 years (30). Consistent with the results of the study by Gürlek et al., Szuck et al. in their study on the men aged more than 60 years also showed that IGF-1, IGFBP-3 and IGF-1/IGFBP-3 index were not correlated with BMD, bone mineral apparent density (BMAD) or bone size. However, in men aged 19-60 years, IGF-1 was positevely correlated with BMD and BMAD of total hip and with cortical thickness of femoral neck (20). Johansson et al. showed that serum concentrations of IGFBP-3 were reduced in men with IO. However, there were no significant linear correlations between histomorphometric indices and IGFBP-3 levels (31). Rucker et al. showed that serum IGF-1 was positively correlated with serum calcium and 25 OH D and negatively correlated with PTH. However, there was no evidence for a relationship between IGF-1 and serum osteocalcin, albumin, creatinine or alkaline phosphatase (32).

The present study included 112 adult men referring to our outpatient clinic. This type of recruitment cannot exclude the possibility of bias in the results. This should also not be considered an epidemiological study because the distribution of age in the sample does not reflect the age distribution of the Turkish male population. Although these drawbacks exist in the formation of the study protocol, this is nevertheless the first study in the literature performed to determine whether circulating IGF-1 and IGFBP-3 levels were associated with BMD and bone turnover markers in middle aged Turkish men.

In conclusion, serum IGF-1 levels were lower in men with reduced BMD and positively correlated with BMD in the lumbar spine. However, neither IGF-1 nor IGFBP-3 was correlated with bone turnover markers, consistent with previous studies.

Finally, one of the most critical issues is whether circulation IGF-1 concentrations are representative of skeletal IGF-1 concentrations. Studies of these factors in skeletal cells are needed to clarify their role in the pathophysiology of idiopathic male osteoporosis.

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