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Can Urinary Gamma Glutamyl Transferase be Used as a Bone Resorption Marker in Postmenopausal Osteoporosis?

[Üriner gamma glutamil transferaz postmenapozal osteoporozlu olgularda kemik yıkım belirteci olarak kullanılabilir mi?]

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ABSTRACT

Objectives: To evaluate whether γ-glutamyl transferase can be used as a new novel bone resorption marker in postmenopusal osteoporotic subjects**.**

Design and methods: 156 postmenopausal subjects were divided into three groups according to their lumbar spine T-score measured by dual-energy X-ray absorptiometry as normal, (control group, n=56), osteopenic (n=50) and osteoporotic (n=50). Deoxypyridinoline and γ-glutamyl transferase from urine samples and osteocalcin and bone specific alkaline phosphates from blood samples were assessed.

Results: Osteocalcin and bone specific alkaline phosphates levels were increased in osteoporotic group ($p<0.05$). Although there is a tendency to increase in deoxypyridinoline values in osteoporotic group, this difference did not reach to a statistical significance. No significant differences were observed in urinary γ-glutamyl transferase levels between the three groups ($p>0.05$). No significant correlation was found between urinary γ-glutamyl transferase and deoxypyridinoline, bone specific alkaline phosphates and osteocalcin ($p>0.05$). Urinary γ-glutamyl transferase levels showed no significant correlation with neither bone mineral density nor T scores in all subjects ($r= 0.058$ $p= 0.625$, $r=-0.074$ $p=0.533$ respectively).

Conclusions: Our primary findings did not support the suggestion that urinary γ-glutamyl transferase could be used as a potential marker for bone resorption in postmenopusal osteoporotic subjects**.**

Key Words: Osteoporosis, postmenopausal, gamma glutamyl transferase, biochemical markers

ÖZET

Amaç: Üriner γ-glutamyl transferaz enziminin yeni bir kemik yıkım belirteci olup olmadığını araştırmak

Gereç ve Yöntem: 156 postmenapozal olguda çift enerji X-ışını soğurum cihazı ile kemik mineral yoğunluğu ölçümleri yapıldı ve olgular lumbar L2-4 T skorlarına göre normal (kontrol grubu, n=56), osteopenik (n=50) ve osteoporotik (n=50) olmak üzere üç gruba ayrıldı. Bütün olgularda idrar örneklerinden deoxypyridinoline, γ-glutamyl transferaz ve kan örneklerinden osteokalsin ile kemik alkalen fosfataz ölçümleri değerlendirildi.

Bulgular: Serum osteokalsin ve kemik alkalen fosfataz seviyeleri osteoporotik grupda artmış olarak saptandı ($p<0.05$). Deoxypyridinoline seviyeleri osteoporotik grupta osteopenik gruba göre daha yüksek saptanmasına karşın istatistiksel anlamlılık saptanmadı. Çalışma grupları arasında üriner γ-glutamyl transferaz açısından anlamlı farklılık saptanmadı. Üriner γ-glutamyl transferaz'nin deoxypyridinoline, kemik alkalen fosfataz ve osteocalsin ile anlamlı bir ilişkisi gözlenmedi. Tüm olgular ele alındığında üriner γ-glutamyl transferaz düzeyleri ile lumbar bölge kemik mineral yoğunluğu ve T skorları arasında anlamlı bir korelasyon olmadığı saptandı (sırasıyla $r= 0.058$ $p= 0.625$, $r=-0.074$ $p=0.533$).

Sonuç: Bu çalışmanın sonuçları postmenapozal osteoporozlu olgularda üriner γ-glutamyl transferaz'nin kemik yıkımının bir belirteci olarak kullanılabileceği görüşünü desteklememektetir.

Anahtar kelimeler: Osteoporoz, postmenapoz, gammaglutamyl transferase, biyokimyasal belirteçler

Introduction

Osteoporosis is a systemic disease characterized by low bone mass and micro architectural deterioration of bone tissue, resulting in an increased risk of fracture and has reached epidemic proportions [1]. The importance of this situation has stimulated the development of many biochemical markers to assist in evaluating the fracture risk ratio and treatment efficacy [2]. Recent developments in the field of bone markers include; identification of new biochemical markers providing additional information on the complex pathways leading to bone fragility; application of novel technologies such as proteomics for the discovery of novel markers, and refinement of the clinical interpretation of markers [3,4]. There are limitations to the clinical utility of many of these bone markers, but researchers continue to explore ways to improve their clinical use.

The most sensitive markers which have been developed during the last few decades include serum osteocalcin (OC), bone specific alkaline phosphatase (BSAP), which are known as bone forming markers and deoxypyridinoline (DPD), N-terminal propeptide of type I collagen for bone formation (NTX), and the crosslinked C- telopeptides of type I collagen for bone resorption (CTX) which are known as bone resorption markers [5,6]. Such markers of bone turnover can be used to predict the rate of bone loss in postmenopausal women and can also be used to assess the risk of fractures [7]. However, these are all time consuming and costly, so a more simple and inexpensive method is needed.

In recent years, gamma glutamyl transferase (GGT), an ectopeptidase which catalyzes the transfer of gamma glutamyl to an acceptor [8] was also shown to play an important physiological role in bone metabolism through cysteine metabolism [9]. Gamma glutamyl transferase is a widely distributed enzyme and its increase is frequently seen in patients with excess alcohol intake, fatty liver or primary billier cirrhosis [10]. These clinical conditions are also well known as risk factors for osteoporosis. However, the underlying molecular mechanisms between these conditions are still under investigation. In a combined animal and human study, Asaba *et al*. found that urinary GGT levels are significantly increased in osteoprotegerin-deficient osteoporotic mice as well as patients with postmenopausal osteoporosis [11]. These investigators concluded that measurement of urinary levels of GGT can be a simple and useful method for mass screening to identify those with increased bone turnover and hence at increased risk for bone fracture. The aim of the present study was to investigate the usefulness of urinary GGT as a discriminative marker in postmenopausal osteoporotic subjects. To our knowledge, the present study is the first one investigating the relationship between the urinary GGT changes and its relation with other bone turnover markers (DPD, BSAP and OC) systematically in normal, osteopenic and osteoporotic postmenopausal subjects.

Participants and methods

Participants

In this cross-sectional study, 156 postmenopausal subjects who are not on osteoporosis treatment were enrolled in the study in a period of 18 months and divided into three groups according to their T-score measured by dual-energy X-ray absorptiometry (DXA). Subjects with lumbar spine (L2-4) T-score >-1 were included in group 1 (control group, n=56), subjects with T-score between -1 and -2.5 (osteopenia) were included in Group 2 (n=50) and subjects with T-score <-2.5 (osteoporotic) enrolled in Group 3 (n=50). Exclusion criteria were; (i) subjects over 75 years, (ii) subjects receiving treatment for osteoporosis such as estrogens, calcitonin, bisphosphonates, anabolic streroids or vitamin D, (iii) subjects receiving any drug interfering with bone metabolism (iv) subjects with any disease that can interfere with bone metabolism, such as metabolic, inflammatory, hepatic, renal, malignant or immune disorder (v) subjects with premature or surgical menopause and (vi) subjects taking non-steroidal anti-inflammatory drugs and having liver, bilier and pancreas diseases, excessive alcohol intake, diabetes mellitus, and congestive heart failure. All subjects gave their written informed consent to participate in the study, which was approved by the Ethics Review Committee of the University of Celal Bayar, Faculty of Medicine.

Methods

Bone mineral density (BMD) was measured by DXA using a Lunar DPX (IQ, MD, USA) at the following sites; lumbar spine (L2-L4) in AP projection, and the left hip including femoral neck, trochanter, Ward's triangle and total hip. A diagnostic criterion for osteoporosis was proposed according to the WHO criteria and was defined as T-score of less than -2.5 SD**.** Osteopenia was defined as a T-score between -1 and -2.5 SD in any region. Subjects with a T-score higher than -1 SD was accepted as normal [12].

Blood and morning urine samples were obtained after 10 h of fasting. Osteocalcin and bone BSAP were assessed from serum and DPD, GGT and creatinine (Cr) were assessed from urine samples simultaneously in a blinded manner. Urine parameters were corrected for Cr and the results were given as DPD/Cr and GGT/Cr. Serum intact OC were assessed by a solid phase two site chemiluminescent immunometric assay. Urinary DPD levels were assessed by means of enzyme-labeled chemiluminescent immunoassay (IMMULITE, Diagnostic Products Corporation, Los Angeles, CA, USA). Osteocalcin test method minimum detection limit was 0.1 ng/ml; intra- and inter- assay coefficient of variation (CV) % was 2.8 and 3.9 respectively at 6.2 ng/ml. Reference range for OC levels was 2-22 ng/ml. Deoxypyridinoline test method minimum detection limit was 4.4 nM; intra- and inter- assay CV% was 8.9 and 9.7 respectively

at 100 nM. Reference range for DPD levels was 3.0-7.4 nM DPD/mmolCr. Serum BSAP levels was assessed by Enzyme-Linked Immunosorbent Assay (ELISA) method (Metra, Hannover, Germany). Reference range for BSAP levels was 14.2-42.7 U/L. BASP test minimum detection limit was 0.7U/L; intra- and inter- assay CV% were 5.8 and 5.2 respectively at 12U/L. Urine GGT and Cr were measured by an enzymatic endpoint spectrometric method (Beckmann Coulter, DXC800 analyser, Beckmann Coulter Galway, Ireland). GGT test serum and plasma minimum detection limit was 5.0 U/L; intra- and inter- assay CV% were 3.5 and 5.3 respectively at 85.7 U/L. Creatinine urine reference range was 0.8-2 g/24 hours. Urine Cr test minimum detection limit was 10 mg/dl; intra- assay CV% was 2 for level 10 mg/dl and and inter-asay CV % was 3 at level 100 mg/L. DXA data were handled as gold standard in the diagnosis of osteopenia or osteoporosis and sensitivity and specificity of the urinary GGT was calculated as ''true positive / true positive+ false negative'' and ''true negative / true negative+ false positive'' respectively.

Statistical analysis

SPSS version 11.0 (Chicago, IL, USA) was used for statistical analysis. Data were expressed as mean ±standard deviation (SD). Statistical differences among the groups were identified with One-Way analysis of variance (ANOVA) followed by the Bonferroni post hoc test. Chi-square test was used to compare the categorical variables. Pearson's rank correlation coefficient was used to investigate the correlations between the individual changes in both evaluated bone resorption parameters. *P* values of less than 0.05 were considered to be significant.

Results

Demographic findings of the subjects included in the study were summarized in Table 1. Age, number of pregnancy, body mass index and total lactation period after birth (year) were similar in all groups. Mean duration after menopause in years was significantly higher in the osteoporotic group (Group 3) ($p=0.03$). BMD values and T-score at L2-4 were significantly higher in the osteopenia and osteoporotic groups compared to the control group ($p<0.0001$, $p<0.0001$, respectively).

Differences in bone turnover markers and urinary GGT in all groups of postmenopausal subjects were given in Table 2. Osteocalcin and BSAP levels were found to be significantly increased in the osteoporotic group compared to osteopenic and control group ($p=0.025$, $p=0.001$ respectively). Although there is a tendency to increase in DPD values in the osteoporotic group, this difference did not reach to a statistical significance ($p=0.062$) (Table 2). No significant differences were observed in urinary GGT levels between the study groups (0.377±0.188 U/mmolCr , 0.386±0.198 U/mmolCr, 0.406±0.25 U/mmolCr in Group 1, 2 and 3 respectively, ($p=0.91$).

Correlation analysis only revealed a significant correlation between BSAP and OC ($r=0.379$, $p=0.001$), No significant correlation was found between urinary GGT and other bone formation markers in all postmenopausal subjects included in the study ($p>0.05$) (Table 3). The resorption markers however, urinary GGT and DPD revealed non-significant correlation ($r= -0.290$ $p= 0.24$, $r= 0.106$ $p=0.665$, $r= 0.279$ $p=0.128$ in Group 1, 2 and 3 respectively).

When a cut-off value of 0.45 U/mmolCr (40 U/gr Creatinine) was taken for urinary GGT (as Asaba *et al.*), the calculated sensitivity and specificity for discriminating those with osteoporosis was found as 32% and 41% respectively. When a cut-off value of 0.40 U/mmolCr was taken for urinary GGT which is the mean of the osteoporotic group, the calculated sensitivity and specificity for discriminating those with osteoporosis was found as 32% and 41% respectively. The statistical analysis (Pearson's Correlation) between urinary GGT and BMD (gr/cm2) or T-score also revealed that there was no significant correlation in all subjects included in the study ($p>0.05$) (Table 3).

Discussion

Although the presence of some experimental and animal studies [13,14], human studies evaluating the relationship between the GGT and osteoporosis are very limited [11]. In an extensive Medline literature search we could only find one study Asaba *et al*. In this combined animal and human study, Asaba *et al*. found that urinary GGT significantly increased in osteoprotegerin-deficient osteoporotic mice as well as patients with postmenopausal subjects [11]. Investigators concluded that measurement of urinary levels of GGT could be a simple and useful method for mass screening to identify those with increased bone turnover and hence those at increased risk for bone fracture. Moreover, these investigators also concluded that there was a strong correlation between urinary GGT excretion and DPD levels. They reported that, when a cut-off value of 40 U/gCr (0.45 U/mmolCr) was taken for urinary GGT, the calculated sensitivity and specificity for discriminating those with elevated bone resorption was found as 61% and 92% respectively.

In the present study, we investigated the validity of this suggestion. One way of testing the validity of a marker is to investigate its negative correlation with the formation markers and positive correlation with the resorption markers in normal, osteopenic and osteoporotic subjects. To the best of our knowledge, this is the first study investigating the relationship between the urinary GGT changes and its relation with other bone turnover markers systematically in normal, osteopenic and osteoporotic postmenopausal subjects. However, the results of the present study did not support the suggestion that urinary GGT could be used as a potential marker for bone resorption in postmenopausal subjects which was supposed by Asaba *et al* in a combined experimental and human study [11]. Our results showed that urine GGT

Table 1. Characteristics of all groups and differences between the groups with One -Way ANOVA test

	Group 1 (n =56) (Control)	Group 2 (n =50) (Osteopenia)	Group 3 (n= 50) (Osteoporosis)	p
Age (years)	57.9±7	60.0±8.7	61.6±7.2	0.15
BMI (kg/m2)	29.3± 3.9	28.7± 4.3	27.3± 3.2	0.09
Pregnancy				
0-2	11 (%37)	9 (%30)	13 (%28.3)	0.61
3 or more	19 (%63)	21 (%70)	33 (%71.7)	0.44
Mean duration after menopause (years)	10.45±9.2	11.5±7.6	16.3±8.7	0.03
Duration of total Lactation time (year)	2.53±1.6	4.8±4.8	3.32±3.2	0.10
BMD (g/cm²) (L2-4)	1.12±0.2	1.04±0.3	0.81±0.1	0.0001
T- score (L2-4)	0.28±1.1	-1.25±1	-2.78±0.6	0.0001

BMI; body mass index. BMD; bone mineral density

Table 2. Bone turnover markers and urinary GGT levels in all groups of postmenopausal subjects.

	Group 1 (n =56) (Control)	Group 2 (n =50) (Osteopenia)	Group 3 (n= 50) (Osteoporosis)	p
BSAP (U/L)	42.4±17.2	68.4±22.6	112.8±37.4*	0.001
OC (ng/ml)	11.6±8.4	16.6±12.5	29.2±13.5*	0.025
DPD (nmol/ mmolCr)	8.03±1.6	9.8±2.3	14.5±4.8	0.062
Urinary GGT (U/ mmolCr)	0.377±0.2	0.386±0.2	0.406±0.3	0.910

* statistical difference compared to control group
Differences between the groups were investigated by One-Way ANOVA test (Bonferroni post hoc test)
GGT; gamma glutamyl transferase
BSAP; bone specific alkaline phosphatase
OC; Osteocalcin
DPD; deoxypyridinoline
Cr; creatinine

Table 3. The correlations between urinary GGT and bone turnover, BMD, T-scores of all subjects included in the study.

	All subjects (n =156)	
	r	p
BSAP (U/L) and Urinary GGT(U/mmolCr)	-0.078	0.51
OC (ng/ml) and Urinary GGT(U/mmolCr)	0.13	0.27
DPD (nmol/ mmolCr) and Urinary GGT(U/ mmolCr)	0.11	0.35
BMD L2-4 and Urinary GGT(U/mmolCr)	-0.027	0.82
T- score and Urinary GGT(U/mmolCr)	-0.21	0.08

The correlations were performed with Pearson's rank correlation coefficient test
BMD; bone mineral density

levels were very similar in all groups of postmenopausal subjects and no significant correlation was found between GGT and DPD, BSAP and OC. In our study, when a cut-off value of 0.45 U/mmolCr (40 U/gCr) was taken for urinary GGT, the calculated sensitivity and specificity for discriminating those with osteoporosis was found at 32% and 41% respectively, which were relatively low percentages compared to Asaba *et al*'s results (a cut-off value of 40 U/gCr (0.45 U/mmolCr) sensitivity and specificity as 61% and 92% respectively) [11].

The conflict between the two studies may be explained by the differences in study design. Asaba *et al* found a very strong relationship for urine GGT excretion in postmenopausal osteoporosis [11]. However they only included 10 postmenopausal subjects with documented BMD with DEXA. The remaining 551 postmenopausal subjects included in their study had no DEXA data to determine if they were normal, osteopenic or osteoporotic. In our opinion, post-menopausal subjects should be classified according to their DEXA data to make a more clear comment in terms of the relationship between urine GGT and DPD levels. Moreover, GGT is a microsomal enzyme that has been shown to be excreated in many tissues such as renal, liver and bilier systems. GGT has also been shown to be elevated in many conditions including pancreas diseases, excessive alcohol intake, diabetes mellitus, congestive heart failure and NSAID usage [15]. In our study, we tried to exclude all of these factors while Asaba *et al* did not mention any exclusion criteria in their study.

In the past decade, animal and cell culture experimental studies have shown that gamma glutamyl transferase (GGT), which catalyzes the transfer of gamma glutamyl to an acceptor, plays an important physiological role in bone metabolism through cysteine metabolism [9]. In a recent experimental study, Hiramatsu *et al* suggested that GGT plays a regulatory role in octeoclast development which does not require the enzyme activity and may represent a novel mode of action as a cytokine [13]. Moreover, mice deficient in GGT were shown to exhibit growth retardation and severe osteoporosis [16]. Osteopenia in GGT-deficient mice is associated with decreased bone formation, which can be treated with supplemental N-acetylcysteine [9]. This suggests that GGT plays an important physiological role in regulating bone formation through cysteine metabolism**.** The work by Levasseur *et al* coupled with the work presented here indicates that either a deficiency or an excess of GGT may result in osteoporosis. Clearly osteoporosis is exerted through distinct mechanisms including suppression of bone formation due to systemic deficiency in enzymatic activity, and an acceleration of bone resorption by an excess of GGT. Both of these mechanisms function independently of enzyme activity and reflect local effects [13].

The effect of Receptor Activator Factor-kB Ligand (RANKL) on bone metabolism and its critical role for the regulation of bone remodeling has been demonstrated [17]. Multiple clinical trials are currently in progress to investigate the therapeutic potential of RANKL inhibition by different agents. Although cumulative evidence for the relationship between RANKL and osteoporosis in humans is abundant, data concerning the relationship between GGT, RANKL, and bone metabolism is quite limited [18]. Experimental studies have been performed to investigate further this relationship. The data indicate that GGT may act as an enhancer for RANKL, and stimulate it independent of its own enzymatic activity [14,18]. Further, the research indicates that GGT serves as a pathological bone-resorbing factor [14,18]; however both of these findings are needed to be supported with human studies.

As previously mentioned, increased level of serum GGT was frequently seen in patients with excess alcohol intake and hepatic diseases [19,20] which are frequently accompanied with osteopenia and osteoporosis [21,22] However, the underlying molecular mechanisms between these conditions could not be established yet and are still under investigation. The results of limited numbers experimental studies suggest that GGT may play a significant role in underlying mechanisms between these conditions. In an experimental study performed to illuminate this interaction, Niida *et al*, suggested that osteoclast formation by GGT may account for osteoporosis induced by these hepatic diseases and they demonstrated that recombinant human GGT as well as purified GGT from rat kidney stimulates bone resorption [18].

The main limitation of the present study is the low number of postmenopausal subjects included in the study. So that, newly designed prospective studies with large number of post-menopausal subjects are necessary to confirm our findings.

In conclusion; researches are ongoing for new biomarkers that are less time consuming, inexpensive and practically useful for evaluating the risk ratio of fracture or treatment efficacy in individuals with low bone mass or high bone turnover such as postmenopausal state. Urinary GGT has recently been supposed that it could be used as a potential marker for bone resorption. However, the results of the present study did not support this suggestion for our groups of post-menopausal subjects and no significant correlation was found between urinary GGT and other bone turnover markers. These results should be considered as preliminary against the use of urinary GGT as a bone resumption marker.

Conflict of interest

Authors do not have any conflict of interest.

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