Measurement of Osteocalcin as a bone turnover marker in women before & after menopause in Tikrit city.

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Abstract

Osteocalcin is a small protein (49 amino acids) mainly synthesized by mature osteoblasts, remains the most specific marker of osteoblastic activity. Serum osteocalcin values are higher in children than in adults, rising during puberty and decreasing to adult levels. Most studies show a higher concentration of osteocalcin in adult males than in adult females, but there are no data available regarding the levels in osteocalcin during human ageing. The aim of the study is to measure serum osteocalcin as a bone turnover marker in women before & after menopause in relation to steroid hormones in Tikrit city. The study included 95 female subjects who attended to Dejla hospital for measurement of bone mineral. Fifty subjects from the total 95 subjects are postmenopausal women. Subjects distributed according age into four subgroups ;- Fourteen women in group 1 aged less than forty years, 27 subjects in group 2 aged 40-49 years, 27 subjects in group 3 aged 50-59 years, & 27 subjects in group 4 aged 60 years & above. Serum osteoclacin was measured by the lectrochemiluminescence immunoassay (ECLIA) is intended by use on Elecsys and Cobas (e411 (Roch Device) immunoassay analyzers. There is no significant difference in regard to serum osteocalcin concentration between group aged 40-49 years (15.63 ± 6.54 ng/ml) as compare to age group less than 40 years (16.229 ± 4.7 ng/ml). However, there is significant increase in serum osteocalcin concentration in group aged 50-59 years (20.95 ± 13.6 ng/ml) as compare to age group less than 40 years (16.229 ± 4.7 ng/ml). Also, there is significant increase in serum osteocalcin concentration in group aged 60-69 years (18.06 ± 7.32 ng/ml) as compare to age group less than 40 years (16.229 ± 4.7 ng/ml). There are fifty postmenopausal women, they have a significant increase in serum osteocalcin concentration (23.32 ± 14.1 ng/ml), as compare to age group less than 40 years (16.229 ± 4.7 ng/ml) & group 40-49 years (15.63 ± 6.54 ng/ml). The present study shows that there is significant increase in serum osteocalcin concentration associated with increase in age of women.

Key words: Osteocalcin, estrogen, ALP, Women.

Introduction

Osteocalcin is another important bone turnover marker. Which is a major bone matrix protein with high affinity for hydroxyapatite. This property is conferred by several residues of the calcium-binding amino acid γ-carboxyglutamate (Gla), which requires vitamin K for its biosynthesis, (1).

Osteocalcin is a small protein (49 amino acids) mainly synthesized by mature osteoblasts, remains the most specific marker of osteoblastic activity. Its function has still to be further investigated (2). The majority of osteocalcin secreted

by the osteoblast is deposited in extracellular bone matrix; serum osteocalcin represents the fraction of total osteocalcin that has not been absorbed to hydroxyapatite, (3-4). It is postulated that newly synthesized osteocalcin is released into circulation as intact molecule. Serum osteocalcin values are higher in children than in adults, rising during puberty and decreasing to adult levels. Most studies show a higher concentration of osteocalcin in adult males than in adult females, but there are no data available regarding the levels in osteocalcin during human ageing (5-6).

Because it is rapidly cleared by the kidney, the half life of circulating osteocalcin is short i.e. approximately 5 minutes (6). Osteocalcin may play a role in bone mineralization and in the regulation of bone turnover, following the binding of its carboxyglutamic acid residues to hydroxyapatite (7). In untreated postmenopausal osteoporotic women, osteocalcin levels have been shown to correlate with the risk of an osteoporotic fracture, (8-9). Osteocalcin acts as a hormone in the body, causing beta cells in the pancreas to release more insulin, and at the same time directing fat cells to release the hormone adiponectin, which increases sensitivity to insulin, (10).

Current data suggests a possible role of osteocalcin in male fertility. Research suggest that osteocalcin may enhance the synthesis of testosterone, which is a hormone believed to regulate aspects of male fertility (6).

Osteocalcin synthesis is known to be modulated by Vitamin D. Since Vitamin D deficiency remains unrecognized over a long period of time, it may be appropriate to monitor both Vitamin D and osteocalcin levels in patients at risk of developing osteoporosis. Serum osteocalcin levels correlate well with iliac crest histomorphometry and calcium kinetic data. Measurement of decarboxylatedosteocalcin has been shown to be a good predictor of hip fracture in elderly women. Serial measurements of osteocalcin levels have been shown to be an excellent marker to assess long term effects of antiresorptive therapy, (10).

Osteocalcin, with or without and ALP, could be a useful diagnostic tool to select patients with probable femoral neck, L1-4 spine, or L2-4 spine osteoporosis for BMD measurement, (10). Biochemical bone markers are non-invasive and less expensive diagnostic tools that are beneficial for diagnosis and treatment follow-up of metabolic bone diseases. In addition, while BMD measurements reflect the static status of bone tissue, biochemical bone markers show the dynamic status, (7-9).

Therefore, using BMD measurements together with these markers can make the diagnosis, risk evaluation, and therapy of OP more effective (5).

Osteocalcin can also be decreased in

patients with low bone turnover such as some patients with osteoporosis in renal failure and patients with adynamic bone. OC is a particularly sensitive marker of corticosteroid effects on osteoblasts and is markedly decreased in patients receiving acute high dose steroid, (4).

The aim of the study is to measure serum osteocalcin as a bone turnover marker in women before & after menopause in relation to steroid hormones in Tikrit city.

Subjects & Methods

The study included 95 female subjects who attended to Dejla hospital for measurement of bone mineral. The subjects were divided into four age groups: subjects less than 40; subjects 40 - 49 years; 50- 59 years & subjects above 60 years.

The patients who excluded in this study were patients with secondary osteoporosis as in:- Thyroid diseases, parathyroid problems, hypocalcaemia, vitamin D deficiency & renal diseases.

The blood sample was taken by 10 ml syringe and collected in plane tube then the blood sample centrifuged for 15 minutes for complete separation of serum. The serum samples is separated in 3 plain tubes and stored in -20 °C until assayed.

Serum osteoclacin was measured by the electrochemiluminescence immunoassay (ECLIA) is intended by use on Elecsys and Cobas (e411 (Roch Device) immunoassay analyzers, (13-14).

All data were presented as mean & standard deviation (SD). Unpaired T test was used to compare between variables. P value less than 0.05 & 0.01 were accepted as significant values.

Results

Ninety five women were participated in this study. Table 1 shows the mean & standard deviation of body weight, height & body mass index (BMI) of all subjects. Fifty subjects from the total 95 subjects are postmenopausal women. Subjects distributed according age into four subgroups;- Fourteen women in group 1 aged less than forty years, 27 subjects in group 2 aged 40-49 years, 27 subjects in group 3 aged 50-59 years & 27 subjects in group 4 aged 60 years & above.

Table 2 shows the concentration of serum osteocalcin, estrogen, cortisol & alkaline phosphtase enzyme respectively. Group of women of less than 40 years regarded as control with other groups.

Table 2 shows there is significant reduction in serum estrogen concentration in group aged 40-49 years (68.07 ± 49.06 pg/ml) as compare to age group less than 40 years (167.48 ± 16.23 pg/ml). There is 59.4% of reduction in serum estrogen in group 40-49 years as compare with age group less than 40 years.

Table 2 shows a significant reduction in serum estrogen in women aged 40-49, 50-59 & women above 60 years as compare with women at age group less than 40 years.

Also, there is significant increase in serum ALP in women aged 50-59 & women above 60 years as compare with women aged less than 40 years.

Also, table 2 shows In the present study, there are fifty postmenopausal women, there have a significant increase in serum ALP enzyme concentration in postmenopasul women (105 ± 26.9 IU/L) as compare to age group less than 40 years (77.33 ± 15.8 IU/L).

The present findings show that there is increase in serum concentration of ALP associated with increase in age.

In table 3, There is no significant difference in regard to serum osteocalcin concentration between group aged 40-49 years (15.63 ± 6.54 ng/ml) as compare to age group less than 40 years (16.229 ± 4.7 ng/ml).

However, there is significant increase in serum osteocalcin concentration in group aged 50-59 years (20.95 ± 13.6 ng/ml) as compare to age group less than 40 years (16.229 ± 4.7 ng/ml).

Also, there is significant increase in serum osteocalcin concentration in group aged 60-69 years (18.06 ± 7.32 ng/ml) as compare to age group less than 40 years (16.229 ± 4.7 ng/ml).

There are fifty postmenopausal women, they have a significant increase in serum osteocalcin concentration (23.32 ± 14.1 ng/ml), as compare to age group less than 40 years (16.229 ± 4.7 ng/ml) & group 40-49 years (15.63 ± 6.54 ng/ml).

The present study shows that there is significant increase in serum osteocalcin concentration associated with increase in age of women.

Discussion

In the present study, there is significant increase in serum osteocalcin concentration in group aged 50-59 years (20.95 ± 13.6 ng/ml) as compare to age group less than 40 years (16.229 ± 4.7 ng/ml).

Also, there is significant increase in serum osteocalcin concentration in group aged 60-69 years (18.06 ± 7.32 ng/ml) as compare to age group less than 40 years (16.229 ± 4.7 ng/ml).

Osteocalcin a small protein mainly synthesized by mature osteoblasts, remains the most specific marker of osteoblastic activity, (1-4). Serum osteocalcin values are higher in children than in adults, rising during puberty and decreasing to adult levels as compare with adults, (5-6).

In untreated postmenopausal osteoporotic women, osteocalcin levels have been shown to correlate with the risk of an osteoporotic fracture. Osteocalcin is secreted solely by osteoblasts and thought to play a role in the body's metabolic regulation and is pro-osteoblastic, or bone-building, by nature. It is also implicated in bone mineralization and calcium ion homeostasis (6-8).

There are fifty postmenopausal women, they have a significant increase in serum osteocalcin concentration (23.32 ± 14.1 ng/ml), as compare to age group less than 40 years (16.229 ± 4.7 ng/ml) & group 40-49 years (15.63 ± 6.54 ng/ml).

The present study shows there is significant increase in serum osteocalcin concentration associated with increase in age of women.

Osteocalcin synthesis is known to be modulated by Vitamin D. Since Vitamin D deficiency remains unrecognized over a long period of time, it may be appropriate to monitor both Vitamin D and osteocalcin levels in patients at risk of developing osteoporosis. Serum osteocalcin levels correlate well with iliac crest histomorphometry and calcium kinetic data. Measurement of decarboxylatedosteocalcin has been shown to be a good predictor of hip fracture in elderly women. Serial measurements of osteocalcin levels have been shown to be an excellent marker to assess long term effects of antiresorptive therapy (6-8).

Biochemical bone markers are non-invasive and less expensive diagnostic tools that are beneficial for diagnosis and treatment follow-up of metabolic bone diseases. In addition, while BMD measurements reflect the static status of bone tissue, biochemical bone markers show the dynamic status. Therefore, using BMD measurements together with these markers can make the diagnosis, risk evaluation, and therapy of OP more effective, (4-6).

Osteocalcin can also be decreased in patients with low bone turnover such as some patients with osteoporosis in renal failure and patients with a dynamic bone. OC is a particularly sensitive marker of corticosteroid effects on osteoblasts and is markedly decreased in patients receiving acute high dose steroid, (9).

There was a significant correlation between osteocalcin and IL-10 ($p<0.05$) in play a role in the pathogenesis of postmenopausal osteoporosis. There was significant increase serum IL-8 ($p<0.001$) and osteocalcin ($p<0.05$) in patients group than control group, (11).

Recent studies have suggested that the increase in bone resorption induced by estrogen deficiency in postmenopausal osteoporotic women, which is at least partly, mediated by increased paracrine production of bone resorbing cytokines (2). IL-1 is one of the most potent stimulators of bone resorption and IL-6 appears to be a potent osteotropic factor that may play an important role in diseases characterized with increased bone resorption (12-13).

The previous results concluded that the TRAP catalytic activity in serum, as well as OC concentration measurement, together with other biochemical markers of bone turnover, can be useful in the diagnosis of osteoporosis, (14). On the other hand, our results agree well with the

previous worker who found increased OC levels in 30% of the patients with primary osteoporosis, (15).

On the basis of results obtained throughout the present study, it can be concluded that, together with other biochemical markers of bone turnover, determination of the catalytic TRAP activity and osteocalcin concentration in blood sera can not only improve the diagnostic potential of osteoporosis, but also be useful in its distinction from osteopenia, (15-16).

Osteocalcin concentrations are influenced by age, gender, and diurnal variation (17-19). Osteocalcin exhibits a diurnal variation with a nocturnal peak, dropping by as much as 50% to a morning nadir. Concentrations are higher in children. With the highest concentrations observed during periods of rapid growth. Males have somewhat higher concentrations of osteocalcin, (20-22). Osteocalcin concentrations have been reported to increase, decrease, or remain unchanged with advancing age, a probable consequence of the heterogeneity of circulating osteocalcin and differences in immunoassay specificity, (23-26).

Elevated levels of serum osteocalcin may be associated with increased activity of osteoblast. Osteocalcin levels are generally increased during menopause. Increased levels of osteocalcin have been reported in patients with high bone turnover osteoporosis and fractures, (25). Verit et al. 2006 studied and found that serum osteocalcin levels in ostmenopausal osteoporotic women were significantly higher than in premenopausal non-osteoporotic women, (27).

In osteoporotic women, deficiency of calcium may lead to lowering of formation of hydroxyapatite crystals. Thus, in the state of decreased rate of bone mineralization, free osteocalcin may be available for circulation in the blood, (27). This may explain the increased concentration of osteocalcin in the serum of osteoporotic postmenopausal women. Pino et al. 2005, found that osteocalcin is a promising marker of bone turnover useful in the diagnosis and follow-up of high turnover osteoporosis, (26). Similar observations were reported by a number of other studies, (14, 28-29).

In osteoporosis, Brown et al. found that serum osteocalcin level correlated well with histological markers of bone formation rate. Serum osteocalcin has also been reported as being predictive of the rate of bone loss after menopause. As a tool for selecting the appropriate treatment and as a measure of the response to estrogen replacement therapy, (30).

Assessment of BMD nowadays, is the standard criteria for diagnosis and evaluation of osteoporosis. But BMD provide a static picture of skeleton whereas, the biochemical markers (osteocalcin) of bone turnover can provide dynamic status of bone remodeling and

rapid measurement of skeletal metabolism, (31). The major advantage of using osteocalcin as a clinical index of bone turnover is its tissue specificity and it's relatively low within person's variation. Thus osteocalcin is a specific, sensitive, promising and currently used marker for better prognosis of osteoporosis and for monitoring responses to antiresorptive therapy, (20).

Estrogens are essential for bone maturation and mineralization in both men and women. Direct action of estradiol on osteoclasts decreases the development and the activity of osteoclasts and increases the activity of osteoblasts. Estrogen deficiency induces increased generation and activity of osteoclasts, which perforate bone trabeculae, reduce their strength and increase fracture risk. The life span of functional osteoclasts and thus the amount of bone that osteoclasts resorb may also be enhanced following estrogen deficiency. This suggests that estrogen may prevent excessive bone loss by limiting the life span of osteoclasts and promotes apoptosis of osteoclasts(27,67).

Estrogen has also been shown to regulate the secretion of osteoprotegerin, and inhibit osteoclast differentiation. Estrogen has an important role in male bone homeostasis and estrogen receptor in bone (ERα□□ activation has resulted both in preserved thickness and trabecular number, (32). Many causes lead to estrogen deficiency in women: congenital estrogen deficiency, estrogen resistance due to inactivating mutation in the estrogen alpha receptor gene, aromatase (the enzyme that catalyzes androgens conversion into estrogens) deficiency, and androgen deficiency(27,67).

The present study conclude that measurement of urinary and serum osteocalcin may provide important insights into the metabolic derangements in osteoporosis and other bone disorders.

Also, the present study recommend the followings;-

1-Routine measurement of osteocalcin in premenopausal women.

2- Osteocalcin synthesis is known to be modulated by Vitamin D. Since Vitamin D deficiency remains unrecognized over a long period of time, it may be appropriate to monitor both Vitamin D and osteocalcin levels in patients at risk of developing osteoporosis.

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Table 1 shows the mean & standard deviation of age, body weight, height & Body mass index (BMI) of all subjects (95 subjects)

Parameters	Less Than 40 years (14 patients)	40-49 years (27 patients)	50-59 years (27 patients)	Above 60 years (27 patients)	Postmenopause (50 patients)
Age	30.16 ± 6.5	44.68 ± 2.59	54.04 ± 2.5	67.15 ± 8.1	59.6 ± 7.3
Weight (Kg)	72.91 ±15.1	78.64 ± 13.9	85 ± 11.7	80.04 ± 17.6	81.7 ± 11.3
Height (Cm)	158.4 ± 6.1	155.95 ± 4.1	157.3 ± 6.3	155.6 ± 5.4	157.3 ± 5.7
BMI (Kg/M^2)	29.05 ± 6	32.3 ± 5.45	34.5 ± 6.9	33 ± 6.8	32.6 ± 6.2

Table 2 shows bone mineral density parameters, ALP, osteocalcin, cortisol & Estrogen E2 in all studied groups.

Parameters	Less than 40 years (12 patients)	40-49 years (22 patients)	50-59 years (24 patients)	Above 60 years (25 patients)	Postmenopause (50 patients)
Osteocalcin (ng/ml)	16.229± 4.7	15.63 ± 6.54	20.95± 13.6	18.06± 7.32	23.318± 14.1
Cortisol (nmol/L)	305.48± 141.26	326.73±124.4	369.77± 61.1	326.5± 179.92	323.478± 128.11
E2 (pg/ml)	167.48± 16.235	68.07 ± 49.06	31.96± 15.7	29.72± 9.4	30.1± 9.7
ALP (IU/L)	77.33± 15.8	82.45 ± 33.87	102.4± 27.6	92.16± 11.9	105.74± 26.98

Table 3 shows the mean & SD of serum concentration of osteocalcin

Age group	Number	Mean ± SD of osteocalcin	P value
Less than 40 years	12	16.229 ± 4.7 ng/ml	Control
40-49 years	22	15.63 ± 6.54 ng/ml	NS
50-59 years	24	20.95 ± 13.6 ng/ml	0.01
Above 60 years	25	18.06 ± 7.32 ng/ml	0.05
Postmenopausal	50	23.32 ± 14.1 ng/ml	0.01