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ORIGINAL STUDY

Estimation of Some Biomarkers Related to Bone Turnover in Iraqi Women

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ABSTRACT

Osteoporosis is defined as a heterogeneous bone disease that leads to weakening in the structure of bones. Bone structure remains dynamic throughout an individual's life, it undergoes continuous growth and resorption in a specific mechanism called bone turnover. Osteoporosis assumes the perturbation of equilibrium of the growth to destruction towards the destructive away. Bone turnover markers consider as indicators for pathway of bone turning over, it classified into two groups: first group to build the bones, the second group to break it down. By using bio tests and assays, these markers can be evaluated to monitor the activity of the markers also to determine treatment options and efficacy according to this activity. Using these markers in osteoporotic cases can give an advantage for their use in other diseases such as cancer. One hundred Iraqi women participated in this study (50 patients with osteoporosis and 50 as control), Another subgroup included 25 women with type 2 diabetes mellitus (T2DM) and 25 without diabetes. Biochemicals markers were measured in this study vitamin D, Vitamin D-binding Globulin, (VDBG), Calcitriol Receptor (CARE), Bone Gamma-Carboxyglutamic Acid-containing protein (BGLAP). This study aimed to evaluate the effect of bone turnover markers on osteoporosis, our study suggests that diagnosing osteoporotic women by measuring bone markers early through the course of treatment may increase the treatment effectiveness with high quality adjusted life years. DEXA used to get results of Spin Bone Mineral Density and Spine T-score. Results show significant difference in biomarkers p-value ≤ 0.05 and there was correlation between bone turnover markers and severity osteoporosis. Bone turnover parameters act as a good biochemical tool in the treating and monitoring of osteoporosis and this become more popular in the clinical manner. These various parameters could be used to measure the risk of fracture and also treatment determination and effectiveness.

Keywords: Osteoporosis, Postmenopausal women, Turnover bone markers

1. Introduction

Osteoporosis is a disease that has heterogenicity and causes weakness of bone structure. In untreated women with this disorder, rates of bone turnover getting are different in high range. Bone turnover markers can be used for prediction risk of osteoporosis, either fractures or injuries. Increment bone resorption causes lower bone density due to the deterioration structure of bone and lack of supplemental formation. Also, it can measure this deterioration and serve as a supplement to bone mineral density tests, which only assess fracture risk. Increased levels of bone turnover markers signal for reduction bone integrity since newly synthesized bone tissue is less mineralized and has fewer posttranslational modifications. The higher levels of bone resorption markers indicate that the fractures were most likely due to a lower bone density caused by increased bone resorption and signaling osteoporosis. Although research shows quickened bone resorption in a certain ratio of women having osteoporosis, there is usually a wide overlap between healthy and diseased individuals. In this disorder, markers of bone turnover have been proposing that a useful prediction tool for the rate of postmenopausal bone loss and the incidence

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of fractures and this marker is valuable in keeping track of the efficacy of the medication, especially hormone replacement therapy (known as antiresorptive) or calcitonin and bisphosphonates (known as bone-stimulating agents). Cancer is a major risk factor for fractures and bone loss. This is due both by directly effects of cancer cells on the skeleton and to mischievous effects of cancer-specific therapies on bone cells. Marked improvements in survival for many cancers explain that strategies to limit bone loss and reduced fracture risk must be incorporated into the care plans for nearly all patients with cancer. The use of bone turnover markers in osteoporosis can pave method for their uses in other diseases like cancer [1]. Our objective was to evaluate the effect of bone turnover markers in women with osteoporosis, this study suggests that surveillance osteoporotic women by measuring of bone markers going early during the procedure of treatment may enhance the treatment effectiveness with high goodness adjusted life years.

In a study by Rosen et al. [2] illustrate that last fractures in women were related to unusual bone turnover. After adjustment for BMD and age, women having fractures were characterized by low levels of Bone Gamma Carboxyated Glutamic Acid containing Protein (BGLAP), three glutamic acid residues contained in its structure, which subject carboxylation of vitamin K-dependent to form gamma-carboxyglutamate. Once somatic growth subsides, the levels of bone markers returning to a level decrease than those seen during natural puberty and growth. This condition happens during the third decade of life. Practically the levels of these markers stay unchanged until 70 years of age. After that a little increase is usually seen in both formation and resorption markers. In contrast, in the age of menopause, the relation with speeding up in bone turnover, is reflected in a (50–100)% increasement in both bone structure and resorption markers [3].

Vitamin D is an important vitamin for bone validity and immunity. Deficiency of vitamin D can cause skeletal deformities or weakness of bones. In the bloodstream Vitamin D-Binding Globulin (VDBG) is the central transporter of vitamin D metabolites. It regulates the bioavailability of vitamin D in many tissues. The major function of VDBG is to bind and carry vitamin D metabolites, (25-hydroxyvitamin D and 1,25-dihydroxyvitamin D) into the bloodstream, also it transport the essential molecule, vitamin D itself. Beyond this substantial role of vitamin D transportation, the involvement of VDBG in a variety of biological operations highlights its benefit in health and disease handling [4]. The active form of vitamin D is Calcitriol, a fat dissolvable vitamin with its function as antioxidant, its role maintains phosphorous and calcium homeostasis, and give strength to the immune system. Calcitriol exhibit its effect by its bound to vitamin D receptors known as calcitriol receptor (CTRE), which are present in brain, skin, skeletal muscles, parathyroid, heart muscles, pancreas, testes, pituitary, ovaries, and blood cells [5].

Women over 65 years old suffers an osteoporotic fracture in percent 1:3, the popular use of protective treatments like vitamin D, calcium, and estrogens is impractically desired (sun exposure recommended). Bone density currently gives a good method of prognosticate fracture happened in the future but shortage sufficient specificity and sensitivity to reasoning widespread screen [6, 7].

2. Methods

2.1. Study participants

In our study we selected 100 Iraqi women (50 patients and 50 control) aged ranged (50–62) years attending Baghdad Teaching Hospital between the period Jaune and September 2024. All participants were screened by questionnaire form, physical test, and DEXA apparatus for the osteoporosis diagnosis. Patients were divided into 25 diabetic and 25 non-diabetics. The exclusion criteria were conditions that affect the metabolism of bones, like: diseases in the liver, kidney, parathyroid, malignant tumours, menopause or oligomenorrhea before 40 years, ankylosing spondylitis, rheumatoid arthritis, heamatological diseases, or previous pathological fractures.

2.2. Sample collection

Blood samples were collected after an overnight fasting and the separated sera were divided for ELISA and biochemical tests, then stored at 20°C until the test.

2.3. Marker measurements

Enzyme-linked immunosorbent assay (ELISA) is a plate-based assay technique designed for detecting and quantifying soluble substances such as peptides, proteins, antibodies, and hormones. was used for biochemical tests: Vitamin D (Vit.D), Vitamin D-binding protein globulin (VDBG), calcitriol receptor (CARE), bone gamma-carboxyglutamic acid-containing protein (BGLAP). A spectrophotometer was used to estimate: FBS, insulin, and lipid profile. DEXA was used to get results of Spin BMD and Spine T-score. The WHO international reference standard for osteoporosis diagnosis is a T-score of −2.5 or less

Table 1. Age in tow studied groups.

Parameters	Patients n = 50 Mean ± SD	Control n = 50 Mean ± SD	P-value
Age, years	60.58 ± 5.08	50.66 ± 5.56	0.55
Parameters	Patients with T2DM n = 25 M ± SD	Patients without T2DM n = 25 M ± SD	P-value
Age, years	61.58 ± 5.08	56.66 ± 5.56	0.66

Table 2. Biochemical parameters in osteoporotic women and control.

Parameters	Patients n = 50 M ± SD	Control n = 50 M ± SD	P-value
BMI Kg/m^2	34.40 ± 7.59	25.95 ± 5.49	0.007
Spin BMD (g/cm^2)	0.96 ± 0.12	1.06 ± 0.10	0.01
Spine T-score	−2.98 ± 0.55	0.37 ± 0.74	0.001
Vit.D	21.91 ± 2.39	26.23 ± 2.05	0.012
VDBG	0.23 ± 0.06	0.71 ± 0.03	0.05
CARE	0.77 ± 0.25	1.02 ± 0.37	0.14
BGLAP	22.44 ± 2.33	5.34 ± 0.78	0.001

at the femoral neck (FN). Osteoporosis diagnosed in postmenopausal women and men aged >50 years if the T-score of the lumbar spine, total hip or FN is −2.5 or less. Insulin resistance was measured by using the Homeostatic model Assessment (HOMA-IR) using the equation: [HOMA-IR = FBS × FBI/405] [8].

Where: FBI is fasting blood insulin concentration (mU/L)

FBS is fasting blood sugar concentration in (mg/dl)

The results of T.score categorized as follows [9]:

1. If T.score ≤ −2.5 diagnosed as osteoporosis.
2. If T.score −2.5 < T.score < −1 diagnosed as Osteopenia.
3. If T.score > −1 diagnosed as Normal.

Bone Mineral Density values were expressed in g/cm^2, BMD is more than one (standard deviation) SD below the reference mean in osteopenia. However, with osteoporosis, the BMD is 2.5 SD or lower than the reference mean.

Index of Body, mass, (BMI) is measured by using by equation: BMI = weight/(height) ^{2} [10]

BMI = kg/m^2

2.4. Statistical analysis

Calculations of results were done by using the SPSS V17.0 T-test of student was used to get the variance in markers used between postmenopausal women and control on the one hand and comparing diabetic women with non-diabetic on the other hand, results indicate as Mean ± SD. P-value reflects signification at level ≤0.05 and a highly significant at levels ≤0.01.

2.5. Ethical approval

Written approval by: Applied Science Department, Applied Chemistry Branch, University of Technology-Iraq.

3. Results and discussion

Regarding to results in Table 1 it is clear no significant difference appears in age between studied groups [p-value more than 0.05).

Age is a remarkable factor affecting metabolism of bone. The formation of new bone in teenage years and childhood, is going faster than the remodeling of the old bones. The formation of bone outpaces resorption until reaching limit bone mass at 30 years of age. Next which bone resorption in slow mechanism begins to exceed the bone formation, and the hazard of osteoporotic fracture twofold within 7–8 years after 50 of age [11].

The data in Table 2 shows that BMI significantly difference between patients and control groups. This could explain the deteriorated lipid metabolism result from the decrease effect of estrogen in women reach post menopause [12]. Similar to our results, the study of Barbagallo et al. [13] they summarize the relation between obesity and hormonal disturbance in postmenopausal women. Our results in agreement with previous studies [14, 15]. Two potential mechanisms have been proposed to explain how BMI affects osteoporosis. The first mechanism involves mechanical loading, where additional weight imposes higher static mechanical stress on bones. This stress can then trigger adaptive responses, leading to changes in bone quality and structure. Heavy individuals

tend to attain higher peak BMD in early adulthood, which exerts a greater load on weight-bearing joints and results in higher BMD, reducing the likelihood of osteoporosis in old age. The second mechanism involves the physiological function of adipose tissue, which influences bone through an endocrine pathway. Adipose tissue impacts bone metabolism by metabolizing sex steroids, indirectly protecting against bone loss. Adipose tissue expresses and secretes adipocytokines such as leptin and adiponectin. Leptin stimulates osteoblast proliferation, mineralization, collagen synthesis, and inhibits bone resorption, while adiponectin promotes excessive bone resorption associated with bone loss, negatively affecting BMD, particularly in postmenopausal women. Current evidence indicates that leptin positively affects BMI, while adiponectin is negatively associated with BMD, making it a relevant adipokine negatively linked to postmenopausal osteoporosis.

Our data revealed a significant ($p \leq 0.05$) decrease in Spin-BMD in patients when compared to control, Table 2. BMD is a good tool for evaluation activity of osteoclast cell. Hormonal change leads to reduce BMD and this is clarified that fast bone resorption after the latest menses. Losing of bone reaches a maximum level at (3–4) yea. after onset of menopause, then gradually declines for a few years and at the end bone loss per year remains about (1–1.5) % [16].

The lumbar spine BMD is in general considered the best position for determined treatment-related effects in the management of osteoporosis [17] In this study, the average value of T-score was significantly difference with control (p-value = 0.001) The definition of WHO to osteoporosis as having a BMD lower than the value 2.5 SD, that mean (T-score ≤ -2.5) under the average BMD of a younger female. If osteoporosis is caused by fractures that are relevant to the disease, it is diagnosed as severe or established osteoporosis [18, 19].

Bone turnover is constantly occurring. Alteration in the proportion of bone turnover possibly affect the quality of bone. Considering the constraints of BMD and the properties of bone turnover markers that clarify bone quality, attention must be given to the potentially important role of these markers for prognosticate fracture risk and choosing valuable treatment [20] Our results revealed that vitamin D appears significantly lower in patients compared to control p-value = 0.012. In his study on the influence of vitamin D on osteoporosis, Tidaporn et al. [21] reported that vitamin D have a major role in the stimulation of bone matrix synthesis and maturation, increases the efficiency of osteoclasts and affects bone cell differentiation [22] conducted a case–control study in which they evaluated blood vitamin D levels in fractured patients and compared to controls, they discovered that there was lower in patients, also found that vitamin D deficiency was related to a higher risk of fracture. This increment in fracture hazard was not only independent of the frequency of falls, but also of physical action, kidney function, fragility and levels of steroid hormone, and was mediated in part by raised bone resorption. As a result, serum 25(OH)D levels of 20 ng/ml have been associated with an increased risk of hip fractures.

Additional actions attributed to VDBG include potential direct mechanism on bone resorption. A study of Charlotte et al. [23] points to relationship between VDBG levels and bone density by regulate of calcium metabolism, which is important for keeping healthy bones, and having anti-cancer characteristic in the form of the vitamin D-binding protein macrophage-activating factor.

Vitamin D utilizes its biological effects via its metabolite CARE, and vitamin D can diffuse quickly through the membranes of cells and bind to CARE and form heterodimers with the vitamin D response elements to control the transcription of genes. The VDR also regulates the transcription of genes by reacting with other nuclear receptors [24]. Osteoporosis in postmenopausal women can still be without any signs or symptoms for a prolonged time, but the only indication is changes in some biomarkers of bone turnover, these parameters Vitamin D, VDBG, CARE, and BGLAP. Most patients of osteoporosis have related to bone form and resorptive process, but the resorption speed can far exceed the formation protein of BGLAP [25], it is produced during the formation of bones and has a consolidated, calcium-dependent $\grave{\alpha}$-helical shape in which the Gamma Carboxyglutamic Acid (GLA) residues react to form hydroxyapatite in the matrix of bone and simplify absorption. Bone mineralization happened in this manner. In osteoporotic women, Phosphorus and calcium deficits reduce the growth and formation of hydroxyapatite crystals, which allow free BGLAP to circulate in the blood. This could explain the reason that BGLAP levels are higher in the sera of osteoporotic women.

Fasting blood sugar appears in diabetic women in a significant way p-value = 0.01 increase compared with control. Fasting glucose is correlated with osteoporosis incidents [26].

Results revealed in Table 3 that T2DM patients have normal BMD and the difference was non-significant when compared with control, according to some data [27]. This explains additional benefits of metformin (the main first-line medication for the treatment of T2DM) therapy, like improved bone quality and reduced risk of fracture it also has an important role by reducing hepatic synthesis of glucose in

Table 3. Markers between diabetic and nondiabetic women.

Parameters	Patients n = 25 M ± SD	Control n = 25 M ± SD	P-value
FBS mg/dl	180.96 ± 73.4	95.29 ± 15.79	0.01
BMI Kg/m^2	31.77 ± 6.86	25.66 ± 7.24	0.122
Spin BMD (g/cm^2)	0.97 ± 0.18	0.99 ± 0.15	0.26
Spine T. score	−1.1 ± 1.31	−0.74 ± 1.41	0.29
HOMO-IR	2.67 ± 1.29	1.28 ± 0.33	0.001
Vit.D ng/ml	23.29 ± 1.92	25.23 ± 1.55	0.11
VDBG ng/ml	0.34 ± 0.12	0.86 ± 0.13	0.04
CARE ng/ml	1.09 ± 0.31	0.66 ± 0.41	0.011
BGLAP ng/ml	9.99 ± 6.5	4.54 ± 1.12	0.001

Table 4. Lipid profile in patients and control.

Lipid profile	Patients n = 50 M ± SD	Control n = 50 M ± SD	P-value
TC (mg/dl)	185.34 ± 40.02	178.95 ± 41.08	0.47
TG (mg/dl)	166.93 ± 95.62	155.61 ± 82.11	0.39
HDL (mg/dl)	66.54 ± 12.07	76.43 ± 7.00	0.04
LDL (mg/dl)	113.19 ± 13.25	125.76 ± 38.99	0.094
VLDL (mg/dl)	33.38 ± 12.02	31.12 ± 8.30	0.04

blocking the major gluconeogenesis enzymes, and enhances peripheral insulin sensitivity. Investigation has shown that it has a good effect on growth of bone.

Zoulakis *et al.* [28] reported that T2DM is related to an increased risk of fracture, our results show that there was a significant difference in vit.D metabolites (VDBG and CARE) as shown in Table 3, the causes for the increased fracture hazard in women with T2DM are still not clear but some hypotheses suggest that T2DM destroyed various bone properties that have a worse effect on increasing fracture risk. The gathering of advanced glycation end products in bone has been suggested to be responsible fragility of bone in diabetic women. Other pathways have been proposed and involve impaired bone turnover, many epigenetic regulators, increased levels of sclerostin or altered bone marrow fatty cells. Illness duration, bone quality impairment, and osteopenia, all of which can raise the risk of bone fragility and fractures, are likely to be the reasons [29] Moreover, BGLAP titers could be increased in postmenopausal women having deficiency of vitamin D, these results were agreed with our results, while in a study conducted by wang *et al.* [30] found that BGLAP level in T2DM postmenopausal women were lower than control which disagree with our results.

Results of the lipid profile in Table 4 revealed that there was a significant enhancement in HDL, LDL, and VLDL in postmenopausal women compared with control. Because steroid hormone receptors, especially estrogen and androgen are present in visceral and subcutaneous adipose tissue, endogenous gender hormones have been shown to change lipid profiles in those women. As a result, alterations in endogenous female hormone levels in middle-aged women's adipocytes may disrupt lipid metabolism [31].

The results in the present study refer to dyslipidemia in osteoporotic women, this can be explained by several mechanisms. Firstly, for starters, the nuclear hormonal receptor Peroxisome Proliferator Activated Receptor and this will involve in the connection between lipid biomarkers and BMD. Lipid metabolites have the ability to activate PPARγ. When PPARγ levels increase, osteogenesis is reduced, which results a greater bone loss. Secondly, high levels of lipids, especially TG levels, are linked to more oxidized lipids and higher levels of oxidative stress. The increment of oxidative stress may both impede osteoblast differentiation and enhance adipocyte development. Thirdly, high blood TG levels are linked to increased bone marrow fat, resulting in lowering trabecular BMD [32].

These findings are consistent with the study of [33]. Low HDL concentrations have been linked to an increased risk of osteoporosis, but increase LDL levels considered as a risk factor.

Sun et al. revealed in their study that 86.98% of diabetic postmenopausal females have dyslipidemia. In these results there was a significant difference in (HDL, LDL, and VLDL) when comparing diabetic osteoporotic women with control, this result were consistency with the study of Azad et al. who showed significant differences in TC, TG, HDL, and VLDL between studied groups [34].

Table 5. Lipid profile in diabetic and non-diabetic patient.

Lipid profile	Patients n = 25 M ± SD	Control n = 25 M ± SD	P-value
TC (mg/dl)	234.95 ± 66.08	185.34 ± 40.02	0.037
TG (mg/dl)	299.61 ± 82.23	155.93 ± 95.62	0.034
HDL (mg/dl)	43.43 ± 7.58	67.54 ± 12.07	0.004
LDL (mg/dl)	122.76 ± 38.46	113.19 ± 13.25	0.084
VLDL (mg/dl)	59.922 ± 9.14	31.18 ± 13.34	0.008

Table 6. Correlation between parameters.

Correlation Within Studied Factors		Person Correlation	Sig (2-tailed)
Insulin	BMI	0.224	0.033
Vit.D	CARE	0.835	0.001
Vit.D	FBS	0.275	0.014
Vit.D	LDL	−0.238	0.02
Vit.D	VLDL	−0.218	0.004
VDBG	FBS	0.780	0.01
VDBG	TC	−0.562	0.01
VDBG	LDL	−0.579	0.01
VDBG	Spine T score	0.634	0.03
TC	TG	0.34	0.001
TC	HDL	0.082	0.01
TC	LDL	0.407	0.001
TG	HDL	−0.301	0.004
TG	LDL	0.259	0.01
TG	VLDL	0.407	0.001
TG	Spine T score	0.262	0.01
TG	Spine BMD	0.250	0.02
HDL	VLDL	−0.334	0.001
HDL	Spine T score	0.242	0.02
HDL	Spine BMD	0.215	0.04
LDL	VLDL	0.575	0.001
LDL	Spine T Score	−0.381	0.001
LDL	Spine BMD	−0.268	0.01
LDL	BMI	−0.232	0.03
VLDL	Spine T score	−0.309	0.003
VLDL	Spine BMD	−0.286	0.01
Spine T score	Spine BMD	0.795	0.001
Spine T score	BMI	0.327	0.002
BMD	BMI	0.263	0.01

3.1. Correlations between parameters

Correlations between parameters illustrated in Table 6.

As shown in the Table 6, the correlations between the parameters are weak(except Vitamin D with CARE), although the p-value showed a significant change, as the results showed that the correlation coefficient value was less than 0.05.

4. Conclusion

In Iraqi women Bone turnover markers (Vitamin D, VDBG, CARE, and BGLAP) increased after menopause and this relates to spine T-score bone loss, these markers could be used to diagnose osteoporosis in those women. Markers of bone turnover are considered as an important biochemical parameter in the monitoring and treatment strategy of osteoporosis are becoming more common in the clinical manner. These various markers can be used to evaluate fracture risk as well as treatment determination and efficacy. Early diagnosis of osteoporosis using bone turnover markers may enhance treatment effectiveness and improve quality-adjusted life years (QALYs).

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