Estimation of Serum Osteocalcin Levels in Osteoporotic Postmenopausal Iraqi Women with Type 2 Diabetes Mellitus

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Abstract:

Background: Osteoporosis (OP) is a systemic disease characterized by low bone mass and micro architectural deterioration of bone tissue, resulting in an increased risk of fractures and has touched rampant proportions. Osteocalcin, one of the osteoblast-specific proteins, showed that its functions as a hormone improves glucose metabolism and reduces fat mass ratio. This study is aimed to estimate the osteocalcin and glucose level in blood serum of osteoporotic postmenopausal Women with and without Type 2 Diabetes.

Materials and methods: 60 postmenopausal women with osteoporosis divided into two groups depending on with or without T2DM, 30 patients for each. Serum samples of 30 healthy postmenopausal women were collected as control group. Osteocalcin was measured by ELISA method using a kit of (CUSABIO. China). Glucose was determined by spectrophotometric method.

Results: Mean serum osteocalcin in postmenopausal osteoporotic women without Type II Diabetes is higher than control group and the group with T2DM ($p \le 0.001$). **Conclusion**: Bone formation marker increases at postmenopausal osteoporosis women; Hyperglycemia also induces osteoblast function and reduces of production osteocalcin at T2DM.

Key words: Osteocalcin, Glucose, Osteoporosis, T2DM.

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 touch rampant proportions [1]. According to WHO, 30% of postmenopausal women and 70% of women aged above 80 are affect by osteoporosis [2].

Osteoporosis is one of the health problems arising in women entering menopausal age, and will presumably seriously affect the lives of postmenopausal women. Osteoporosis the potentially and serious consequences of osteoporotic fracture increase with advancing age of the population, making skeletal health assessment an important component of routine care in postmenopausal women [3].

Diabetes mellitus is a common disease in most parts of the world [4]. It affects skeletal system and bone metabolism through multiple pathways and it has been recognized as a major risk factor for osteoporosis [5].

Several mechanisms have been proposed for diabetes-related osteoporosis. Poor glycemic control can induce hypercalciuria which has been considered a potential risk factor for osteoporosis in patients with type 2 diabetes [6].

Osteocalcin (OC), Osteocalcin, also known as "bone gamma-

*Department of Chemistry, College of Science for women, Baghdad University, Baghdad, Iraq. **Branch of Clinical Biochemistry, College of Medicine, Baghdad University, Baghdad, Iraq. carboxyglutamic acid (Gla) protein (BGP)," is the most abundant no collagenous protein of bone matrix [7]. In almost all cases of osteoporosis the resorption rate can far exceed the formation. Osteocalcin is synthesized during the bone formation [8] .Lee et al. showed that OC functions as a hormone that improves glucose metabolism and reduces fat mass, [9].

Clinically, serum OC level is utilized as a bone formation marker, because OC is synthesized by osteoblast Therefore, vitamin-K dependent gamma-carboxylate OC, which binds to hydroxyapatite in bone [10].

The aims of this work is to estimate the osteocalcin and glucose levels in blood serum of postmenopausal osteoporosis women with T2DM, and test the effect of diabetes on osteocalcin level in postmenopausal osteoporosis women with diabetes group in comparison of postmenopausal osteoporosis women without diabetes.

Materials and Method:

This study was conducted from December 2012 to June 2013. Samples were collected from 90 postmenopausal women, which were selected from patients who visited Rheumatology and Rehabilitation Outpatient Clinic in Baghdad Teaching Hospital. Postmenopausal status was defined by the absence menses for more than one year in a woman 50 years of age and over. Patients were divided into two groups. Group A, includes a total of 30 patients (postmenopausal women) complaining from osteoporosis and diabetes with a mean age (61.27±5.67 years). Group B includes a total of 30 patients (postmenopausal women) complaining from osteoporosis without diabetes, with a mean age (62.33 ± 8.41) and Control, this group including 30 apparently healthy controls (postmenopausal women) mean age

 (60.18 ± 6.47) . The phlebotomy protocol specified a blood draw after a 12-h fast for all study participants.

The blood samples were allowed to clot and then sera were separated by centrifugation at 3000 rpm for 10 min at room temperature. Serum was aliquot into storage vials, labeled, and frozen at -20° C, until used for the hormones analyses. Patients were diagnosed as osteoporosis and controls as normal by measuring bone mineral density (BMD), using dual energy xray absorptiometry (DXA) according to World Health Organization (WHO) diagnostic.

T-score -1.0 or greater is "normal".

T-score between -1.0 and -2.5 is "osteopenia".

T-score -2.5 or below is "osteoporosis".

Serum investigations were included Glucose measuring by spectrophotometer and serum osteocalcin measuring by enzyme linked immunosorbent assay (ELISA) using kit manufactured by (CUSABIO. China)[11].

Anthropometrics measurement: weight in kilogram and height in meter were measured for each women and body mass index (BMI) was calculated using the following equation: BMI = weight in kilogram / height in meter² [12]. Fat% was calculated using the following equation: Fat% = (1.20 x BMI) + (0.23 x Age) - (10.8 x gender) - 5.4 [13], where male gender = 1 and female = 0.

Statistical analysis

Statistical analyses were performed using SPSS for Windows (version 17). The results were expressed as the mean \pm S.D. Student ANOVA was used for statistical analysis, P \leq 0.05 were considered statistically.

Results:

[Mean ± SD] of body mass index [BMI], waist circumference [WC] and fat% for the studied groups are shown in Table (1). The results showed that WC of A group is significantly higher than B and control groups, [P < 0.001], Also WC of B group is significantly higher than control groups, [P < 0.01]. The results were not recorded any significant differences of BMI and Fat% in both patient groups in comparison with control.

Table 1: shows Anthropometrics par	rameters of the patient	and healthy groups.
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	Patient groups		Control group
	Group A	Group B	
Characteristic	Osteoporosis patients	Osteoporosis patients	Group C
	with diabetes	without diabetes	[n=30]
	[n=30]	[n=30]	
BMI [Kg/m ²]Mean ±SD	27.83±4.25	26.45±3.05	26.87±1.98
W. C. $[cm]$ Mean \pm SD	$93.77 \pm 18.17^{b,c}$	77.87 ± 7.47^{a}	83.87±3.91
Age (y) Mean ± SD	61.27±5.67	62.33±8.41	60.18 ± 6.47
Fat% Mean ± SD	13.90 ± 4.96	12.01±3.74	12.99± 2.49

Results were expressed as the mean \pm SD.

a P <0.01 compared with control group.

b P < 0.001 compared with control group .

c P < 0.001 compared with group B.

Values of osteocalcin and glucose in group A, group B and group C are shown in Table [2]. The results showed that osteocalcin level in group A is significantly lower than groups B and C.

Table 2: Mean ±SD concentrations of osteocalcin [ng/ml], glucose [mmol/l] in patient and control groups.

	Patient groups		Control group
Characteristic	Group A Osteoporosis patients with T2DM [n=30]	Group B Osteoporosis patients without T2DM [n=30]	Group C [n=30]
Osteocalcin[ng/ml]Mean ±SD	5.706 ±1.337 ^{c,b}	12.708 ± 3.428 ^a	9.401 ± 3.038
F.S.glucose[mmol/l]Mean ±SD	9.39 ±1.23 ^{a, c}	5.09 ± 0.66^{a}	4.70 ± 0.448

Results were expressed as the mean \pm SD.

a : P <0.01 group compared with control group.

b: P <0.001 group compared with group B.

c: P <0.001 group compared with control group.

Table 3: shows the application of simple regression analysis which revealed appositive significant correlation between serum Osteocalsin levels and serum F.S.G for patient groups and control.

Groups	Correlation	P value
Patients group A	0.374	0.00001
Patients group B	0.352	0.00001
Controls	0.458	0.00001



Fig.1: Correlation of serum Osteocalsin level with serum F.S.G for patients Group A



Fig. 2: Correlation of serum Osteocalsin level with serum F.S.G for patients Group B



Fig.3: Correlation of serum Osteocalcin level with serum F.S.G for Control group.

Discussion:

study revealed This that serum osteocalcin level in postmenopausal women was significantly higher than control group. This explains that the elevated level of serum osteocalcin may be associated with activity of Osteocalcin levels osteoblast. are generally increased during menopause [14]. Increased levels of osteocalcin have been reported in patients with high bone turnover osteoporosis and fractures [15]. Osteocalcin has a high affinity for calcium and exhibits a compact- calcium dependent a-helical conformation, in which the Gla (bone gamma-carboxyglutamic acid) residues binds and promote absorption to hydroxyapatite in the bone matrix, in this way mineralization of bone takes place [16].

Deficiency of calcium in bone may lead to lowering the formation of hydroxyapatite crystals. Thus, in the state of decreased rate of bone mineralization, free osteocalcin could be available for circulation in the blood. This may explain the increased concentration of osteocalcin in the serum of osteoporotic postmenopausal women [17].

The increased levels of osteocalcin in post-menopausal the group are attributed to an estrogen deficiency, a finding which correlates to those of similar studies [18], which indicate a clear correlation between the estrogen deficiency and the raised osteocalcin The decreased estrogen levels. concentrations at the menopausal ages lead to a lower intestinal absorption of resulting in low serum calcium. calcium concentrations and an increased osteoclastic resorption of the bone. Both cases are leading to increase the bone turnover, thereby contributing as risk factors for the development of osteoporosis [19]. Serum (OS) levels were lower in women have osteoporosis and T2DM, reported that osteocalcin levels were founded to be lower in subjects with T2DM than in healthy people. In Another study the serum osteocalcin levels were analyzed in patients with type II diabetes and found to be lower in those with poor metabolic control in comparison to those with good metabolic control and the healthy groups [20]. Figure 1 shows the distribution of osteocalcin in serum of



the studied groups.

Fig. 4: distribution of Osteocalcin [ng/ml] in A group, B group and Control group.

In-vitro	and	in-vivo	studies	were
demonstr	ated	that	hypergly	cemia

suppresses osteoblast functioning [21], and, thereby, decreased production and secretion of osteocalcin in T2DM.

Hyperglycemia also induces increase production of reactive oxygen species (ROS), which are responsible for bone marrow ageing. However, the increasing of glucose concentration may directly be toxic to osteoblast [22].

In conclusion, osteocalcin availability in serum of postmenopausal osteoporotic women without diabetes is higher than its availability in healthy and postmenopausal osteoporotic women with diabetes. The osteoporosis is a silent disease, thus the value of osteocalcin in osteoporotic patients may be useful as a marker for this type of diseases.

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تقدير مستوى الاوستيوكالسين في مصول النساء العراقيات المصابات بهشاشة التدير مستوى الاوستيوكالسين في مصول النساء العراقيات المصابات بهشاشة

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الخلاصة:

المعلومات الاساسية: هشاشة العظام مرض يصيب الجهاز العظمي الذي يتميز بانخفاض كتلة العظم واعتلال التركيب الهندسي الدقيق لنسيج العظم، الذي يمكن ان ينتج في زيادة خطر نسب استتشراء الاصابة بالتكسرات للعظام. وجد ان الاستيوكالسين الذي يعد احد الهرمونات المتخصصة للخلايا البانية للعظم ، يعزز من ايض الكلوكوز ويقلل من نسبة كتلة الدهن. استهدفت هذه الدراسة تحديد مستوى الاوستيوكالسين والكلوكوز في مصول دم النساء بعد سن الياس المصابات بهشاشة العظم ومرض السكري او المصابات بالهشاشة فقط.

المواد وطرائق العمل: قسمت 60 مريضة بهشاشة العظم الى مجموعتين على اساس مجموعة مصابة بالهشاشة والسوري نوع II ومجموعة مصابة بالهشاشة فقط دون السكري وبواقع 30 مريضة لكل مجموعة. تم جمع نماذج من مصول الدم له 30 مراة صحية كمجموعة ضابطة. تم قياس الاستيوكالسين بواسطة الايلايزا وباستعمال العدة التشخيصية (CUSABIO. China) فيما استخدمت الطريقة الطيفية في تحديد الكلوكوز.

النتائج: لقد وجد ان معدل الأوستيوكالسين في مصل دم مجموعة النساء المصابات بهشاشة العظم بدون السكري $p \ge p$ يكون اعلى بالمقارنة مع مجموعة المصابات بهشاشة العظم والسكري معا والمجموعة الضابطة بمستوى $p \ge p$ (0.001).

الاستنتاج: ازدياد دالة تكوين العظم عند النساء المصابات بالهشاشة ، كذلك يحث فرط السكر من وظيفة الخلايا البانية للعظم ويختزل من انتاج الاوستيوكالسين في حالة الاصابة بمرض السكري.