Correlations between Serum Interleukins-2,-4 Levels and Some Biochemical Parameters in Iraqi Patients with Osteoporosis

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Abstract:

Background: Osteoporosis is a frequent disease that is manifested by reduced in mineral density and raised in fracture risk. Recent studies have indicated that osteoporosis is caused by composite connections among local and systemic regulators of bone cell function.

Objective: The purpose of this study was to investigate the relationship between interleukin-2, interleukin-4, and some biochemical markers in Iraqi patients with osteoporosis.

Patients and Methods: Forty five osteoporotic patients were incorporated in this study (30 women and 15 men). Serum fasting glucose, lipid profile, alkaline phosphatase activity, calcium, magnesium, interleukin-2, and interleukin-4 were measured in osteoporotic patients and compared them with the control group.

Results: There was a significant increase in serum fasting glucose, lipid profile except high density lipoprotein cholesterol, and serum alkaline phosphatase activity in osteoporotic patients as compared to the control, (P=0.001). Also, there was a significant increase in interleukin-2 and interleukin-4 in osteoporotic patients as compared to the control, (P= 0.01). While there was a decrease in serum calcium and magnesium levels in osteoporotic patients as compared to the control. Also, there was a significant positive correlation between serum alkaline phosphatase activity with interleukin-2 and interleukin-4 levels in osteoporotic patients, (P= 0.01).

Conclusion: Elevated levels of interleukin-2 and interleukin-4 along with some biochemical markers like; serum alkaline phosphatase in osteoporotic patients might include significant functions in the pathogenesis of osteoporosis.

Keywords: Osteoporosis, Interleukin-2, Interleukin-4.

Introduction:

Osteoporosis which is defined based on bone mineral density (BMD), is a skeletal disorder manifested by compromised bone strength. It is induced by an imbalance between osteoblastic bone formation and osteoclastic bone resorption (1). Osteoporosis is manifested by skeletal tenderness and inclination to fracture due to reduction of bone mass and descent of bone micro-architecture (2). It is metabolic bone disease preponderance in both men and women, especially when they become older (3). The incidence of this disease is mainly reliant on metabolic, genetic, nutrition, hormonal factors, and lifestyle (4). Obesity, dyslipidemia, hyperglycemia, and hypertension are factors connected with the incidence of osteoporosis (5).

Dyslipidemia is a risk factor for cardiac complications. Osteoporosis and cardiovascular disease (CVD) usually are viewed as split disease and parallel to extent in their pathophysiological mechanism (6). Osteoporotic subjects are at risk of CVD and stroke having higher atherogenic lipid levels than subjects without this problem (7). Bone turnover markers (BTMs) have been shown to afford important information for the diagnosis and monitoring of metabolic bone disease. A numeral of

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BTMs can now be determined clinically, and are usually separated into two categories as markers of bone formation and markers of bone resorption. Bone formation markers reflect different phases of osteoblast in bone formation, for instance alkaline phosphatase (ALP) (8).

A sufficient intake of calcium (Ca^{+2}) is necessary to maximize and sustain bone density. A calcium poor diet is a major risk influence for osteoporosis, Ca^{+2} is mislaid from the bones owing to menopause and elderly (9). Calcium supplementation, particularly when combined with vitamin D, can lessen the rate of bone loss and fracture in subjects who are deficient in dietary Ca^{+2} such as the frail elderly (10).

The property of Mg^{2+} on obesity are vital to women's health, as women are more expected to be obese than men (11). It is recommended that sphingolipids perform as regulators of extracellular Mg^{2+}. In a low Mg^{2+} state, ceramide synthase is upregulated, adding to liberate of inflammatory cytokines (12). Phosphatidylcholine is implicated in lessening the lymphatic absorption of cholesterol and is a main constituent of high density lipoprotein (HDL), so furthering the significance of Mg^{2+} for maintaining cardiovascular health (13).

Recent studies have related osteoporosis to the immune system and osteoimmunology is now accepted as a new area of study (14). Though, osteoclasts, predisposed by pro-inflammatory cytokines and anti-osteoclastogenic cytokines, can repress such activity (15). Interleukin-2 (IL-2) has

vital roles in key functions of the immune system, tolerance and immunity, mainly via its direct effects on T cells (16). Interleukin-4 (IL-4) is a 19-kDa pleiotropic type I cytokine, IL-4 an essential immune cytokine that regulates role of lymphocytes and macrophages, in addition to regulates osteoclastogenesis and bone resorption (17). Therefore, this study aimed to examine the relationship between IL-2, IL-4, and some biochemical markers in Iraqi patients with osteoporosis.

Patients and Methods:
The present work was carried out at Medical City Hospital during the period from May 2016 until the end of November 2016. Outpatients were cautiously segregated by taking into account patients' history of osteoporosis in the family and some other factors which may affect the bone turnover markers. A total of 45 numbers of patients (30 women and 15 men) were selected for the study while remaining 45 apparently healthy subjects (23 women and 22 men) were selected as controls. Fasting serum glucose (FSG), lipid profile including [total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C), and calculated low density lipoprotein cholesterol (LDL-C)], ALP activity, Ca^{+2}, and Mg^{+2} were measured using a chemical analyzer in the medical laboratories. Cytokines levels were determined in sera via a sandwich immunoassay kit provided by Human IL-2 and IL-4.
A monoclonal antibody specific for IL-2 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known IL-2 concentrations, control specimens or unknowns are pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for IL-2 are simultaneously incubated. After washing, the enzyme Streptavidin-HRP, that binds the biotinylated antibody is added, incubated and washed. A TMB substrate solution is added which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored

Product is directly proportional to the concentration of IL-2 present in the samples.

Statistical Analysis: Values are provided as mean ± SD. Variances between two groups were tested using t-test. A *p*-value ≤ 0.05 was considered significant.

Results:
Anthropometric characteristics of the studied groups are shown in table (1). No significant difference in age between osteoporotic patients and the control was found, while there was a significant increase in body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP) in osteoporotic patients as compared to the controls, (P =0.01).
Table (2) shows the laboratory characteristics of the studied groups. There was a significant increase in FSG, TC, TAG, LDL-C, and ALP activity while a significant decrease in serum HDL-C was found in osteoporotic patients as compared to the controls, (P =0.001). Also, there was a significant increase in serum IL-2 and IL-4 in osteoporotic patients as compared to the controls, (P =0.01). There was a decrease in serum Ca^{+2} and Mg^{+2} levels in osteoporotic patients as compared to the controls, but it was not significant. Correlations coefficient between serum ALP activity and other variables in osteoporosis group in table (3) showed that there was a significant positive correlation between serum ALP activity with FSG, TC, TAG, and LDL-C, (P= 0.001). Also, there was a significant positive correlation between serum ALP activity with IL-2 and IL-4, (P= 0.01). While, there was a significant negative correlation between serum ALP activity and HDL-C, (P= 0.001).

Table 1: Anthropometric characteristics of the studied groups

Parameter	Osteoporosis (n= 45)	Control (n= 45)	P value
Female/Male	30/15	23/22	-
Age (years)	56.30 ± 8.54	55.01 ± 2.49	0.08
BMI (kg/m^2)	33.01 ± 3.85	25.0 ± 1.56	0.01
SBP (mmHg)	135.48±2.78	124.15 ± 1.24	0.01
DBP (mmHg)	82.27 ± 3.34	74.87±1.85	0.01

Table 2: Laboratory characteristics of the studied groups.

Parameter	Osteoporosis (n= 45)	Control (n= 45)	P value
FSG (mg/dl)	115.18 ± 0.46	84.70 ± 3.34	0.001
TC (mg/dl)	210.70 ± 7.80	124.50 ± 2.80	0.001
TAG (mg/dl)	185.90 ±5.70	75.30 ± 4.90	0.001
HDL-C (mg/dl)	40.30 ± 2.24	52.50 ± 1.60	0.001
LDL-C (mg/dl)	133.20 ± 4.42	56.94 ± 0.22	0.001
ALP (IU/l)	130.42 ± 6.50	65.32 ± 1.20	0.001
Ca^{+2} (mg/dl)	5.22 ± 0.50	10.39 ± 1.45	0.06
Mg^{+2} (mg/dl)	1.0 ± 0.30	2.02 ± 0.40	0.07
IL-2 (ng/ml)	35.30 ± 1.50	28.60 ± 1.20	0.01
IL-4 (ng/ml)	23.58 ± 1.93	15.50 ± 1.21	0.01

Table 3: Correlations between ALP activity and other variables in osteoporosis group

ALP activity	Correlation coefficient (r)
FSG (mg/dl)	0.80**
TC (mg/dl)	0.92**
TAG (mg/dl)	0.98**
HDL-C (mg/dl)	-0.84**
LDL-C (mg/dl)	0.95**
Ca^{+2} (mg/dl)	-0.20
Mg^{+2} (mg/dl)	-0.15
IL-2 (ng/ml)	0.75*
IL-4 (ng/ml)	0.70*

*P = 0.01, ** P = 0.001

Discussion:
Osteoporosis is a developing main public health difficulty with influences on quality and quantity of life that pass medical, social, and economic lines (18). Lifestyle-associated metabolic disorders include disorders of glucose metabolism such as hyperglycemia, hypercholesterolemia, and dyslipidemia (19). Blood glucose level was higher in osteoporotic group than in the controls. Current lines of researches have demonstrated that high blood glucose foremost to impaired osteoblast-mediated bone formation, accelerated bone resorption, microstructural defect, and reduced bone quality (20). Two studies reported the risk of fractures related with lipid levels (21, 22). It has shown that elevated LDL-C and low levels of HDL-C are related with low bone mineral density (23). Management of dyslipidemia requires multidimensional approach like dietary alterations and integration of healthy activity was of central importance in the overall concern plane (24).
The BTMs has influenced the evaluation and treatment of osteoporosis. Elevated levels of bone turnover markers are related with 2-fold increased risk of osteoporotic fracture as well as suggestive to other metabolic bone diseases. The BTMs are significant in management of osteoporosis treatment and thus recognition of optimum treatment for an individual is of primary importance, which could be achieved by estimating bone metabolism (25). Alkaline phosphatase is one of the BTMs, used as a marker of bone metabolism for the management of patients with metabolic bone disease such as paget's disease, hyperparathyroidism, metastatic bone disease, and osteoporosis, ALP might help to guide treatment choices for antiresorptive drugs (26) and also to determine an previous signal of response to treatment usefulness than bone density assessments (27). Many bone diseases affect a demineralization process and therefore many treatments are designed for inhibiting bone resorption. Measurement of a bone resorption marker is thus more pertinent to the assessment of improved bone loss with ailment (28). In this study, a significant increase in ALP activity was found in osteoporotic patients compared to the controlswhich is in accordance with the study of Khan et al., (29). Calcium (Ca^{+2}) is a key constituent of bone (30). In the elderly, particularly among women, Ca^{+2} losses from bone can result in osteoporosis, and it is connected with low levels of circulating vitamin D. There has been much research on dietary supplementation of vitamin D and Ca^{+2} and in the old people, with the goal of minimizing osteoporosis and its complications, such as improved risk of bone fractures (31). Magnesium is a key mineral used for over 300 enzyme functions in the body.It has several approved health claims including its involvement to the maintenance of normal bones. Most Mg^{+2} supplements are solitary sourced, which may not be well absorbed in the gut. A multi-sourced Mg^{+2} supplement is ideal as it will offer an equilibrium of high Mg^{+2} content and good bioavailability (32). Magnesium insufficiency is simply detected with biochemical indications like resistance to vitamin D, low serum Ca^{+2} and Mg^{+2} levels, or clinical symptoms like, muscle cramps, muscle twitching, irregular heartbeat, and elevated blood pressure. It has been postulated that Mg^{+2} is required to prevent osteoporosis in the overall population (33). In the present study, there was a decrease in serum Ca^{+2} and Mg^{+2} levels in osteoporotic patients as compared to the controls. Visceral fat accumulation is related with higher levels of pro-inflammatory cytokines, which may up-regulate receptor activators of nuclear kappa B ligand, leading to increased bone resorption and therefore decreased BMD (34). Though the definite cytokines rise over time, the properties of cytokines are not completely stated. It was revealed that IL-2 has vital functions in the pathogenesis of osteoporosis (35). It have been postulated that IL-2 is a positive regulator which is able of stimulating osteoclastic bone resorption, IL-2 is one of the major causes of decreased bone mineral density and bone formation (36). Interleukin-4 has been shown to act directly on osteoclast precursors and reduce osteoclastogenesis.
Interleukin-4 induced intracellular Ca^{+2} changes, the method by which IL-4 acts on intracellular Ca^{+2} is until now to be determined. These consequences propose that IL-4 acts directly on mature osteoclasts and inhibit bone resorption throughout Ca^{+2} signaling probably by IL-4R-mediated mechanism. It is also reported that IL-4 prevents bone and cartilage damage in collagen-provoked arthritis (37). In the current study, there was a significant raise in serum IL-2 and IL-4 levels in osteoporotic patients as compared to the controls which is similar to the result of Wenxi et al., study (38).

Conclusions:
Elevated levels of IL-2 and IL-4 along with some biochemical markers like; serum ALP in osteoporotic patients might include significant functions in the pathogenesis of osteoporosis.

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