Interleukin-35 in Iraqi's Menopausal Women with Osteoporosis

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Received in: 19 May 2014, Accepted in:5 January 2014

Abstract

Osteoporosis is related to the loss of bone mass that occurs as part of the natural process of aging and in menopausal women.

The aim of this study is to evaluate the levels of interleukin-35 in menopausal women with osteoporosis, that may be used as an early predicted marker of osteoblastic and osteoclastic activity in osteoporosis patients in relation with other diagnostic parameters.

Fasting blood samples were obtained from forty Iraqi menopausal women with mean age of (61.06±6.59)years who were newly diagnosed of osteoporosis by physicians at Baghdad Teaching Hospital as patients group(G1). In addition to , twenty two healthy women were included in the study as a control group(G2) with mean age(60.75±6.35). Serum samples were used for determination of Rheumatoid Factor (RF), Alkaline Phosphatase(ALP), Calcium(Ca^{+2}), Phosphate(PO_4^{-3}) and Interleukin-35(IL-35),while blood sample was used for determination of Erythrocyte Sedimentation Rate (ESR).

The results revealed a highly significant increase in IL-35 level in patients group(G1) compared to the control group(G2) while there are no significant differences in ALP, Ca^{+2} and PO_4^{-3} levels between G1 and G2. The correlation between serum levels of IL-35 and ALP ,Ca^{+2} and PO_4^{-3} in menopausal osteoporosis patients was calculated; A significant positive correlation between IL-35 and ALP was found ($r=0.277, P<0.05$), while there was no significant correlation between IL-35 and each of Ca^{+2} and $PO4^{-3}$ levels ($r=0.191, P>0.05$),($r=0.124, P>0.05$) respectively.

The conclusion obtained from this study that IL-35 may be used as an additional biomarker in predicting osteoporosis in postmenopausal women.

Key Words: Osteoporosis, Interleukin-35

Introduction

Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk[1].Although osteoporosis can occur in men, it is most common in women who have gone through menopause[2].

The interleukin-12 (IL-12) family, is composed of IL-12, IL-23, IL-27, and the newly identified IL-35[3]. The IL-12 cytokine family share binding partners and structural features, there are notable differences in their pattern of expression and secretion. IL-12, IL-23, and IL-27 are all primarily produced by antigen-presenting cells, following their activation by recognition of pathogen-specific patterns. In contrast, secretion of bioactive IL-35 has been described only in regulatory T cells (Tregs)[4-6].

Interleukin-12,IL-23,and IL-27 share the common feature of inducing interferon-production and promoting T-helper 1(Th1) cell differentiation and proliferation, they act differentially on subsets of T cells and with different kinetics. In contrast, IL-35 appears to function solely in an anti-inflammatory fashion by inhibiting T-cell proliferation and perhaps other parameters[3].

The biological effects of IL-12,IL-23,IL-27, and presumably IL-35 are mediated through their interaction with their receptor chains which bind to and induce phosphorylation of the Janus kinase (Jak) family proteins[7]. Jak proteins then phosphorylate tyrosine residues on the intracellular domains of the receptor chains, which act as docking sites for various members of the signal transducer and activator of transcription(STAT) family. Phosphorylated STAT proteins form intramolecular complexes that translocate into the nucleus and bind DNA to initiate the gene expression profile and cellular response that typifies the given cytokine[8,9].

The aim of this study is to evaluate the levels of IL-35 in postmenopausal women with osteoporosis, that may be used as an early predicted marker of osteoblastic and osteoclastic activity in osteoporosis patients in relation with other diagnostic parameters.

Materials and Methods

Fasting blood samples were obtained from forty Iraqi postmenopausal women with mean age of (61.06±6.59) years who were newly diagnosed of osteoporosis by physicians at Baghdad Teaching Hospital as patients group(G1) . In addition to, twenty two healthy women were included in the study as a control group(G2) with mean age of (60.75±6.35). Serum samples were used for determination of RF, ALP, Calcium and Phosphate While Blood sample was used for determination of ESR, using standard procedures of the biochemistry laboratory of the hospital.

Serum IL-35 levels were measured using specific enzyme-linked immunosorbent assay (ELISA) kit (CUSABIO) human IL-35 for *in vitro* quantitative measurement, according to the manufactures protocol[10].

The data were expressed as mean±SD. The comparison between patients and control group were analyzed using student t-test. Pearson's correlation coefficient was used to find the correlation between IL-35 with ALP, Ca^{+2} and PO_4^{-3} in patients group. P-value of < 0.001 and <0.05 were considered highly significant and significant respectively.

Results and Discussion

Descriptive and diagnostic parameters for the two studied groups(G1,G2) are shown in table(1). The results revealed highly significant increase in IL-35, ESR and RF levels in patients group(G1) compared to control group(G2) while there are no significant differences in ALP, Ca^{+2} and PO_4^{-3} levels between G1 and G2.

Calcium is an essential element in the human body and is necessary to many cell functions. Calcium and vitamin D have long been recognized as important and required nutrients for bone health and maintenance. Calcium is a vital component of bone architecture and is required for deposition of bone mineral throughout life. It is the levels of plasma calcium that dictate calcium balance. Adequate intake of calcium is necessary to maintain this balance[11]. The amount of phosphate in the blood affects the level of calcium there.

Calcium and phosphate in the body react in opposite ways: As blood calcium levels rise, phosphate levels fall[12,13].

Alkaline phosphatase (ALP)[EC 3.1.3.1] are a group of enzymes that are present in many different tissues, it is made mostly in the liver and in bone with some made in the intestines and kidneys[14]. It is also made by the placenta of a pregnant woman. The liver makes more ALP than the other organs or the bones. Some conditions cause large amounts of ALP in the blood. These conditions include rapid bone growth (during puberty), bone disease (osteomalacia or Paget's disease), or a disease that affects how much calcium is in the blood (hyperparathyroidism),vitamin D deficiency, or damaged liver cells[15].

The results of the present study are in agreement with previous study that suggested elevated concentration of alkaline phosphatase even levels within the normal range of the enzyme may indicate an increased level of bone cell activity and, therefore, potential bone loss[16].

Bone cell activity is a normal occurrence marked by the breaking down and rebuilding of bone at remodeling sites. When estrogen levels decrease after menopause, the remodeling rate increases, but the process of rebuilding the bone is not as efficient. Estrogen helps to keep calcium in the bones, postmenopausal women are at increased risk for bone loss and osteoporosis[17].

The results in table(2) showed the correlation relation between serum levels of IL-35 with ALP ,Ca^{+2} and PO_4^{-3} in menopausal osteoporosis patients, A significant positive correlation between IL-35 and ALP was noted ($r=0.277, P<0.05$), while there are no significant correlation between IL-35 levels and Ca^{+2} , PO_4^{-3} levels were found ($r=0.191, P>0.05$),($r=0.124, P>0.05$) respectively.

Bone resorption is tightly and dynamically regulated by multiple mediators, including cytokines that act directly on osteoclasts and their precursors, or indirectly by modulating osteoblast lineage cells that in turn regulate osteoclast differentiation. It has been extensively documented that various inflammatory factors produced by activated immune cells act as antiosteoclastogenic factors by different mechanisms. Suppression of osteoclastogenesis by inflammatory factors and cytokines functions as a feedback inhibition system that limits bone resorption and tissue damage associated with infection or inflammation[18].

The role of interleukins in bone metabolism has been investigated, which may help to expose new targets for gene therapy[19].

IL-12 superfamily of cytokines has pleiotropic immune functions with either activating or suppressive roles in various infectious, inflammatory models and
potently inhibits receptor activator of nuclear factor kappa-B ligand (RANKL)-induced human osteoclastogenesis and osteoclastic[20,21].

IL-12 plays an inhibitory role in osteoclastogenesis by directly inhibits osteoclast precursors or by targets other cell types such as stromal/osteoblastic cells or T cells to indirectly suppress osteoclastogenesis [22]. Apoptosis induced by interactions between IL-12-induced Fas ligand (FasL) and Tumor necrosis factor (TNF-α)-induced Fas contributes to the inhibitory mechanisms of IL-12 in TNF-α-induced osteoclastogenesis[23].

The conclusion could be drown from this study that IL-35 could be used as an additional biomarker in predicting osteoporosis in menopausal women.

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Table No.(1): Descriptive and diagnostic parameters for the patients and controls groups.

Parameters / Groups	**Age (year)**	**BMI**	**ESR mm/hr**	**RF IU/ml**	**IL-35 Pg/ml**	**ALP (U/L)**	**Ca^{+2} (mmol/L)**	**PO_4^{-3} (mmol/L)**
Patients Group (G1) N=40	61.06±6.59	30.52 ± 4.37	32.1 ± 3.14	52.10 ± 4.31	35.13 ± 5.01	78.75 ± 13.63	2.35±0.17	1.12±0.26
Control Group (G2) N=22	60.75±6.35	30.10 ± 4.08	10.91 ± 1.31	20.99 ± 6.21	22.13 ± 1.99	73.05 ± 10.75	2.21±0.14	1.22±0.20
P-Value	NS	NS	HS	HS	HS	NS	NS	NS

NS(No Significant) P≥0.05, HS(Highly Significant) P<0.001

Table No.(2): Correlation between serum IL-35 with ALP, Ca and PO_4

Parameters	**IL-35 pg/ml**
ALP (U/L)	r 0.277 P S
Ca^{+2} (mmol/L)	r 0.191 P NS
PO_4^{-3} (mmol/L)	r 0.124 P NS

S (Significant) P<0.05 , NS (No Significant) P≥0.05

انترلوكين-35 عند النساء العراقيات المصابات بهشاشة العظام بعد انقطاع الطمث

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استلم البحث: 19 ايار 2014,قبل البحث في:5 كانون الثاني 2015

الخلاصة

تعود هشاشة العظام الى فقدان كتلة العظم وهو ما يحدث كجزء طبيعي بتقدم العمر وانقطاع الطمث عند سن اليأس في النساء.

تم الحصول على نماذج الدم من اربعين امرأة عراقية مصابة بانقطاع الطمث عند سن اليأس مع هشاشة العظام معدل اعمارهن (61.06±6.59) تم تشخيصهن حديثاً بواسطة الاطباء في مستشفى بغداد التعليمي وعدون مجموعة المرضى (G1). كذلك تضمنت الدراسة (22) امرأة سليمة كمجموعة سيطرة (G2) معدل اعمارهن (60.75±6.35). استعمل المصل في تقدير كل من:

Rheumatoid Factor (RF), Alkaline phosphatase (ALP), Calcium (Ca^{+2}), phosphate (PO_4^{-3}) and Interleukin-35 (IL-35).

في حين تم استعمال الدم في تقدير Erythrocyte Sedimentation Rate (ESR)

اشارت النتائج الى ارتفاع معنوي في مستوى IL-35 عند مجموعة المرضى مقارنةً بمجموعة السيطرة في حين لا يوجد اختلاف معنوي في مستويات ALP , Ca^{+2} , PO_4^{-3} بين مجموعة المرضى ومجموعة السيطرة. كذلك بينت النتائج وجود علاقة موجبة معنوية بين IL-35 و ALP ($r=0.277, P<0.05$) في حين لا توجد علاقة معنوية بين IL-35 وكل من Ca^{+2} و PO_4^{-3} ($r=0.191, P>0.05$),($r=0.124, P>0.05$) على التوالي.

واستنتج من هذه الدراسة ان IL-35 يمكن ان يعتمد كدالة مساعدة في التكهن بالاصابة بهشاشة العظام عند النساء المصابات بانقطاع الطمث في سن اليأس.

الكلمات المفتاحية: هشاشة العظام , انترلوكين- 35