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The Relation between the RANKL and Resistin in Menopausal Women with Osteoporosis

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Abstract. Osteoporosis (OP) is osteometabolic disease characterized by a decrease in bone mineral density(BMD) and due to the deterioration of bone architecture leading to spontaneous fractures. The aim of the current study is to evaluate the biomarkers include the ; receptor activator of nuclear factor NF-κB ligand and Resistin . also measuring the biochemical include: estrogen, calcium, phosphorus, bone mineral density, t-scores in menopausal women with osteoporosis, and further more study the correlation between these biomarkers and biochemical. Method : This study was conducted at DEXA (dual-energy x-ray absorptiometry) Unit in Radiology Department in Al-Sader teaching hospital in AL-Najaf province /Iraq from January2018 to July 2018 to know the prevalence of osteoporosis in Iraqi menopausal women. A total of the (88) women involved in this study, blood samples had been taken from the (68) osteoporosis menopausal women, and from (20) women who were apparently healthy as a control group. Assays of study estimation of the parameters levels that include: Calcium, phosphorous were done by using auto biochemistry analyzer ; Receptor activator of nuclear factor NF-κB ligand bone alkaline phosphatase, Resistin and estrogen, were measurement by using a solid phase enzyme-linked immunosorbent assay (ELISA).Result: The result of study showed a significant decrease ($p< 0.05$) in concentration of serum estrogen, calcium and phosphorus in menopausal women with osteoporosis compared with healthy group. The biomarker revealed a significant increase ($p< 0.05$) in the concentration of receptor activator of nuclear factor NF-κB ligand and Resistin in menopausal women with osteoporosis compared with healthy group. The study exhibited correlation coefficient and showed a significant ($p< 0.05$) negative correlation between BMD and receptor activator of nuclear factor NF-κB ligand. The study showed a significant negative ($p< 0.05$) correlation between estrogen and of nuclear factor NF-κB ligand. This study show significant ($p< 0.05$) positive correlation of receptor activator of nuclear factor NF-κB ligand with resistin.

INTRODUCTION

Osteoporosis is a is term meaning “porous bone”. It is common disease of bones, and causes loss of bone mineral density or insufficient bone formation, making the bones more susceptible to fractures. which may be considered as a silent disease as no symptoms occur in the early stages of the disease [1]. The osteoporosis also define as a common skeletal disorder characterized by reduced of bone mass and deteriorated bone architecture. Which results increased of bone fracture [2] . Osteopenia is define as an imbalance between bone formation and bone resorption resulting in a decrease in bone mineralization, this can further progress to osteoporosis and cause structural failure [3], high bone marrow adipose tissue (MAT) is increased in patients with osteoporosis because MAT is associated with low bone mineral density (BMD) [4] . Two potential mechanisms for the association between MAT and bone. First; mesenchymal stem cells (MSCs) may differentiate into either an adipocyte or an osteoblast .Therefore, a shift in the lineage allocation of the MSCs towards the adipocyte would decrease the number of osteoblasts and thus bone formation. Second; in vitro studies have shown that adipocytes may directly influence osteoblast and osteoclast

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differentiation and function, through secretion of adipokines and free fatty acid [5] . Postmenopausal women considered most cases of osteoporosis due to estrogen deficiency . Fractures of osteoporosis occurs in spine and hip therefore considered high morbidity and mortality [6]. Fractures in osteoporosis affect the muscle and the skeletal systems,cause loss of functional capacity, chronic pain, and compromise quality of life (National Osteoporosis Foundation [7]. The quantity of bone present in the body, bone mineral density, and bone mineral content are parameters using to determine whether a person is osteoporotic [8]. Bone strength is dependent on both the quality of the bone and the quantity of minerals present(BMD). The determinant of bone strength is bone remodeling . Bone quality is a function of bone morphology and architecture as well as of bone material properties [9]. Osteoporotic fractures pose a considerable burden to society, with 42 million of these fractures occurring each year in the United States [10, 11]. Approximately one in every two women and upto1 in every 4 men aged >50 years will have an osteoporotic fracture in their life times [12].The pathogenesis of osteoporosis at the cellular level, communication and coupling between the main bone cell types, the bone-forming osteoblasts and the bone-degrading osteoclasts constitute the smallest functional unit that key molecules conduct the coordinated activities of osteoblasts and osteoclasts during bone remodeling [13]. Histologically, this is apparent by either increase osteoclastic activity or reduced osteoblastic activity. Osteoblasts are rare and little or no new bone apposition can be seen. The cortices are decreased in thickness and cancellous trabeculae become thinned as marrow spaces are widened [14].Cancellous bone becomes porotic more rapidly than compact bone. When osteoporosis is generalized, it tends to be most distinct in the spine and the pelvis [15].

RANKL exists as a homotrimeric protein and is typically membrane-bound on osteoblastic and activated T cells or is secreted by some cells, such as activated T cells [16]. The secreted protein is derived from the membrane form as a result of either proteolytic cleavage or alternative splicing [17] The proteolytic cleavage of RANKL is carried out by matrix metalloproteases (MMP3 or 7) [18] or (A Disintegrin and Metalloprotease domain(ADAM) [19]. Most of the factors known to stimulate osteoclast formation and activity induce RANKL expression by osteoblastic stromal cells. However, RANKL is also highly expressed in lymph nodes, thymus, mammary glands and lung and at low levels in a variety of other tissues, including spleen and bone marrow [20].

The receptor activator of nuclear factor-κB (RANK) and its ligand RANKL, which belong to the tumor necrosis factor (TNF) receptor- ligand family, mediate osteoclastogenesis. The crystal structure of the RANKL ectodomain (eRANKL) in complex with the RANK ectodomain (eRANK) combined with biochemical assays of RANK mutants indicated that three RANK loops (Loop1, Loop2, and Loop3) bind to the interface of a trimeric eRANKL. Loop3 is particularly notable in that it is structurally distinctive from other TNF-family receptors and forms extensive contacts with RANKL. The disulfide bond (C125-C127) at the tip of Loop3 is important for determining the unique topology of Loop3 [21]. RANKL/RANKsignaling not only has a significant role in bone but also functions in other tissues. The system regulates lymph-node formation, mammary-gland development, fever control [22].

Resistin (or 'resistance to insulin') was originally discovered in mice in 2001 and named for its ability to resist (interfere with) insulin action ; Human resistin is a 12.5 kDa cysteine rich peptide with a mature sequence consisting of 108 aa. The human resistin gene is located on chromosome 19 [23], the genes have markedly divergent promoter regions, indicating different mechanisms of regulation, tissue distribution and functions [24,25]. The mature protein has a tendency to form oligomers, thus circulating in human serum in several different low molecular weight and high molecular weight isoforms [26]. Several cell types known to express resistin include adipocytes [27], intestinal epithelium and skeletal muscle cells [28], and possibly astrocytes [29], at that time, it was proposed as a link between obesity and diabetes. Resistin is also known as found in inflammatory zone 3 and adipocyte-secreted factor[30]. Human resistin plays a major regulatory role in the inflammatory response [31], during which macrophages, peripheral blood mononuclear cells (PBMC), and vascular cells are the primary targets of resistin [32]. Resistin up-regulates the expression of pro-inflammatory cytokines such as TNF-α, IL-6, IL-12, and monocyte chemoattractant protein (MCP)-1 in PBMCs, macrophages, and hepatic stellate cells via the nuclear factor-κB (NF-κB) pathway [33].

MATERIAL AND METHOD

This study was conducted in the research from Al-Sader teaching hospital in AL-Najaf province from DEXA unit in the Radiology Department, Fractures and Joints Department and in laboratories of Biology Department/ Faculty of Sciences/ University of Kufa, during the period from. Serum specimens were collected from postmenopausal women patients with osteopenia and osteoporosis in addition to control group .The samples tested were 88 samples which divided to control group were 20 samples, 68 samples from menopausal women patients. The age of menopausal women patients were begin from the 45 year.

Measure the BMD by the DXA

Bone Menial Density (BMD) was recorded at the lumbar spine (L1-L4) (the four lumber vertebrate together), and left and right femurs, by using dual-energy X-ray absorptiometry (DEXA) machine (Dexxum).This score is called the "T-score," and it expresses the bone mineral density in terms of the number of standard deviations (SD) below peak young adult bone mass. Shown in appendix (D, E).

1- **Osteoporosis:** T-score ≤ -2.5 standard deviations below the mean value of peak bone mass.

2- **Severe osteoporosis:** T-score ≤ -2.5 standard deviations below the mean value of peak bone mass plus the presence of at least one fracture.

3- **Osteopenia:** Is a bone mineral density T-score between -1 and -2.5 standard deviations below the mean value of peak bone mass.

Collection of Blood Sample

Blood samples were drawn from vein by sterilized synergies with 5 milliliters. The sample put in the two labeled tubes The gel tube using to allowing clotting the blood in the room temperature for preparation of serum , following using the serum to biochemical , biomarkers and hormonal. Blood was left at room temperature for 10 minutes for clotting, centrifuged 6000 rpm for 15 minutes, and then serum was separated and freezing at -80 ºC until time for performed the laboratory analysis for study.

BIOCHEMICAL PARAMETERS

Assay procedure for Calcium

Specific kit for measuring human Calcium concentrations in serum was supplied by Biolabo SA, France.

Assay procedure for phosphate

This procedure was measured according to the standards required by the manufacturer company

Determination of serum estradiol level

Specific kit of ELISA was using to measuring human E2 concentrations in serum was supplied by CALBIOTECH, China.

BIOMARKER PARAMETERS

Determination of serum receptor activator of nuclear factor-kappa B Ligand (RANKL)level

The level of RANK was determined by using Enzyme-linked immunosorbent assay (ELISA) method, according to procedure provide by the manufacture instructions Elabscience, China.

Determination of serum Resistin level

Resistin (RETN) concentrations in the serum was examined by using enzyme- linked immunosorbent assay (ELISA) according to prepare processed from Elabscience, China.

STATISTICAL ANALYSIS

The well- known statistical system (Graph Pad prism ver. 5) was adopted, and the analysis of variance table one – way anova (by Tukey´s multiple comparisons test) was used for the comparison among subdivided groups in the measured parameters. The results were expressed as (Mean ± Stander Error). The comparison between subgroups was analyzed by t-test. Correlation coefficients were calculated to estimate the correlation between markers and parameters, the descriptive statistics and correlation coefficients were performed by using mega stat (Version v 10.12) for excel 2010 (Motulsky, 2003).

RESULT

The Comparisons of T-scores and Bone Mineral Density in spine and Right Femur from a DEXA Scan; Between the Osteoporosis and Healthy Groups in postmenopausal Women.

The mean values and the standard errors of the BMD and t-scores in spin are shown in the table (1) .Also the mean values and the standard errors of the BMD and t-scores in right femur are shown in the table (2). The results reveal statistically significant differences of postmenopausal women with osteoporosis (PMO) compared with Healthy group , there was significant decreased ($p<0.05$) in BMD and t-scores in (PMO) compared with Healthy group in postmenopausal women.

TABLE 1. The Comparisons of T-scores and Bone Mineral Density in spine From a DEXA Scan; Between the Osteoporosis and Healthy Groups in postmenopausal Women.

Group Aspect	Mean ±S.E. PMO n=68	Mean ±S.E. Healthy group n=20	P-value
BMD	0.8444±0.01402*	1.340 ± 0.03695	<0.05
T-score	-2.201 ± 0.1095*	2.060 ± 0.3293	<0.05

TABLE 2. The Comparisons of T-scores and Bone Mineral Density in right femur from a DEXA Scan; Between the Osteoporosis and Healthy Groups in postmenopausal Women.

Groups Aspect	Mean ±S.E.		P-value
	PMO n=68	Healthy group n=20	
BMD	0.8444±0.01402*	1.340 ± 0.03695	<0.05
T-score	-2.201 ± 0.1095*	2.060 ± 0.3293	<0.05

Determination of Biochemical Parameters and hormonal evidence in serum of patients with Osteoporosis and healthy groups in postmenopausal women.

The results in the table 3 revealed significant differences in minerals and hormonal between PMO and healthy groups, that show significant decrease in the E2, Ca and Pi. The significant was found (the mean values ± the standard

errors) in the serum. The results of figure 1 show a significant decrease ($p<0.05$) in serum E2 concentration in the ages (55-64) and (<65y) than (45-54y) and no significant differences ($p>0.05$) between (54-64y) and (<65y) of PMO.

TABLE 3. The Comparisons of mineral and hormonal evidence between the Osteoporosis and Healthy Groups in postmenopausal Women.

Aspect \ Groups	Mean ±S.E.		p- value
	PMO n=68	Healthy group n=20	
E2 (pg/ml)	15.46 ± 1.747*	22.70 ± 2.687	<0.05
Ca mg/dl	8.19 ± 0.076*	9.15 ± 0.124	<0.05
Pi mg/dl	2.059 ± 0.04423*	2.985 ± 0.1203	<0.05

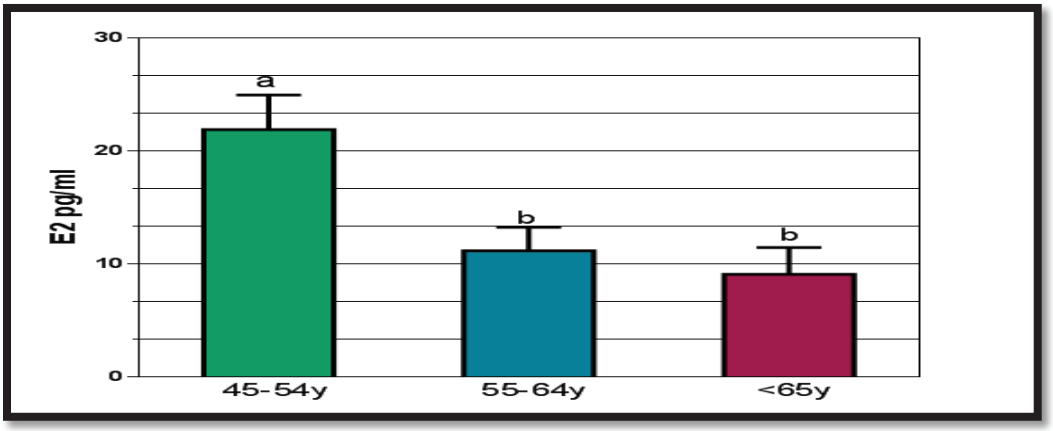

FIGURE 1. Effect of age groups on the serum E2 concentration in postmenopausal osteoporosis. The different letters refer to significant differences ($P<0.05$) between different age groups.

Comparison of biomarkers between osteoporosis and healthy group (HT) in postmenopausal women.

The results in figures 2 and 3 exhibit significant increase ($p<0.05$) in serum levels of RANKL and RETN in POM group compared with in HT group.

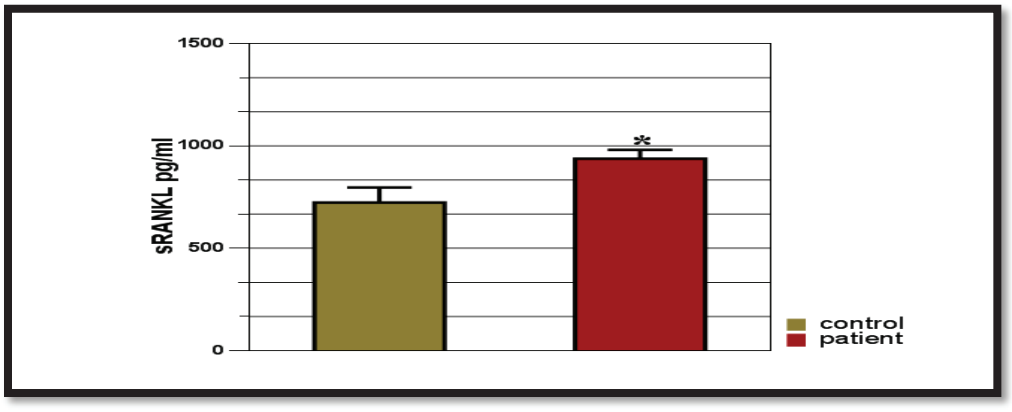

FIGURE 2. Comparison of serum RANKL level between POM and H group in postmenopausal women.

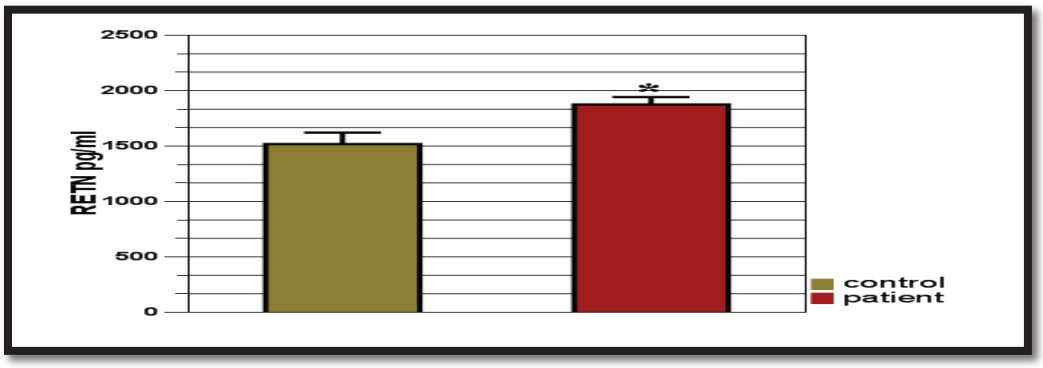

FIGURE 3. Comparison of serum RETN level between POM and HT group in postmenopausal women.

Comparison of biomarkers among different Age groups in patients with osteoporosis in menopausal women.

The result in figure 4 and 5 show no significant increase ($p>0.05$) in serum RANKL and RETN concentration when compare between different age groups in postmenopausal women with osteoporosis.

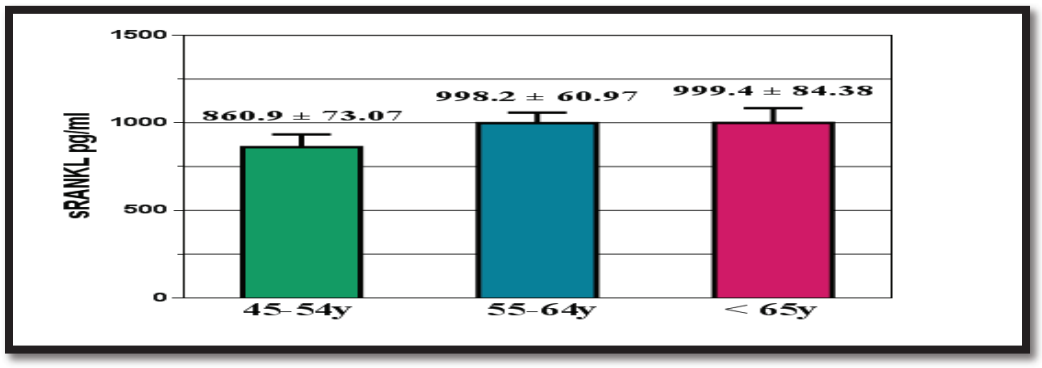

FIGURE 4. Comparison of serum RANKL level at different Ages in POM.

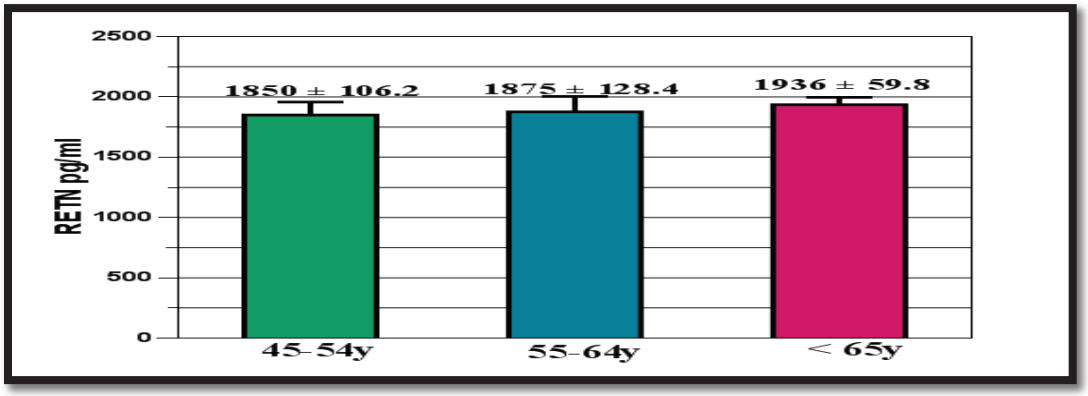

FIGURE 5. Comparison of serum RETN level at different Age groups in POM.

Comparison of biomarkers among different body mass index (BMI) groups in patients with osteoporosis in menopausal women

The results of table 4 indicate there was no significant difference ($p>0.05$) in serum RANKL and RETN concentration at different BMI (normal, over, obese and morbid weight) groups of postmenopausal osteoporosis women.

TABLE 4. Effect of the Body Mass Index on the biomarkers in postmenopausal osteoporosis women.

Groups / Aspect	Mean ±S.E.			p-value
	18-24.9kg/m^2	25-29.9kg/m^2	>30 kg/m^2	
RANKL pg/ml	986.3 ± 83.15	969.0 ± 61.36	887.9 ± 75.14	<0.05
RETN pg/ml	1649 ± 185.1	1906 ± 84.29	1907 ± 111.9	<0.05

Correlation

The study stow in figure 6 indicated, there was a significant negative correlation ($r = -0.43$) between RANKL and BMD concentrations of osteoporosis in postmenopausal women. Figure 7 indicated, there was no significant negative correlation ($r = -0.02$) between RETN and BMD concentrations of osteoporosis in postmenopausal women.

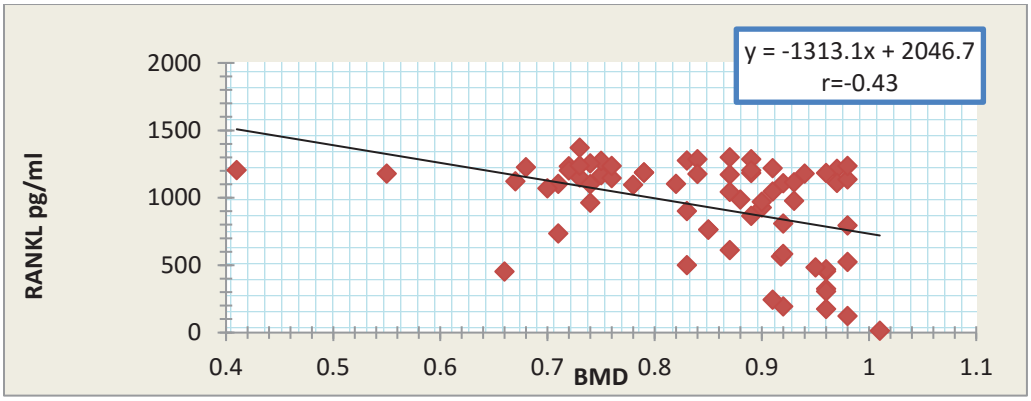

FIGURE 6. Correlation between serum RANKL and BMD levels of osteoporosis in postmenopausal women.

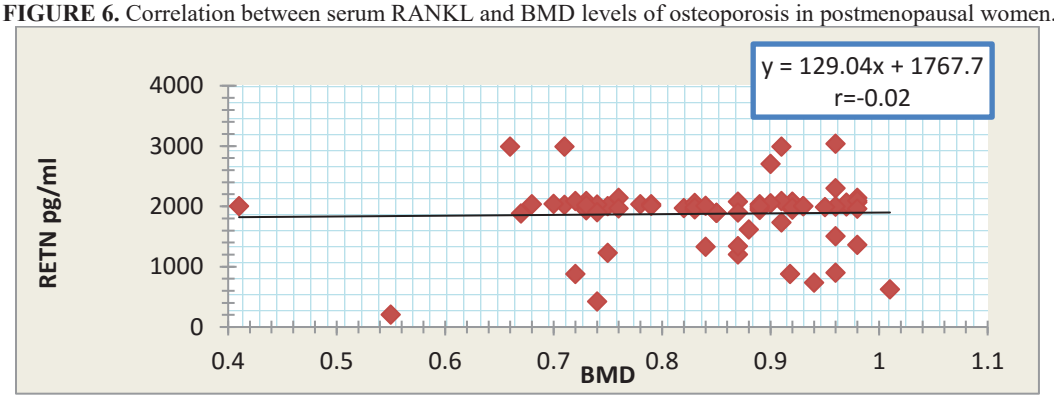

FIGURE 7. Correlation between serum RETN and BMD levels of osteoporosis in postmenopausal women.

In current study show, there was a significant negative correlation (r = - 0.42) of RANKL and RATN with estradiol concentrations of osteoporosis in postmenopausal women, figure 8 and 9.

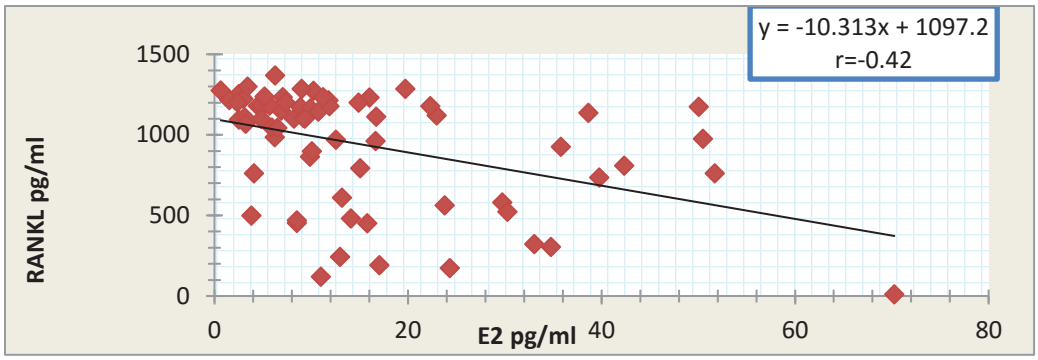

FIGURE 8. Correlation between RANKL and estradiol levels of osteoporosis in postmenopausal women.

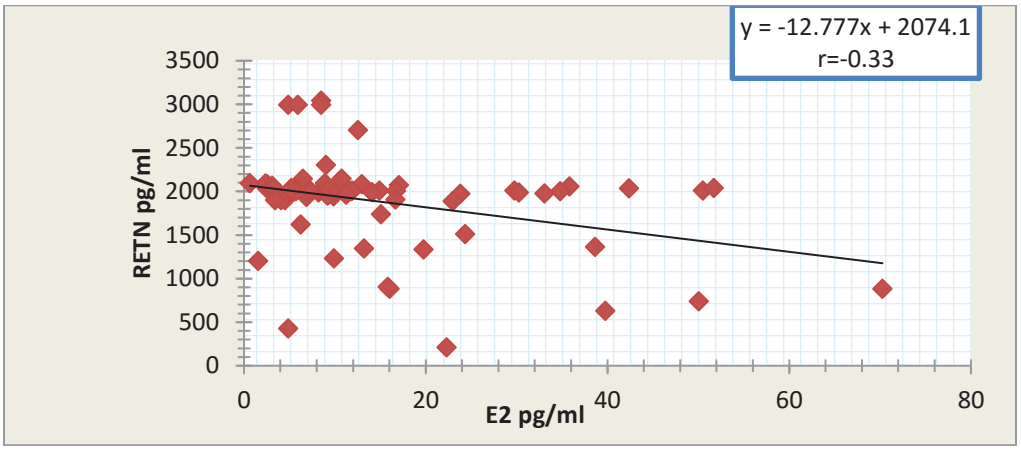

FIGURE 9. Correlation between RETN and estradiol levels of osteoporosis in postmenopausal women.

In the present study show, there was a significant positive correlation (r = 0.28) between RANKL concentrations and age of osteoporosis in postmenopausal women, Figure 10 .also show significant positive correlation (r=) between RANKL and RATN in in menopausal women with osteoporosis, figure 11.

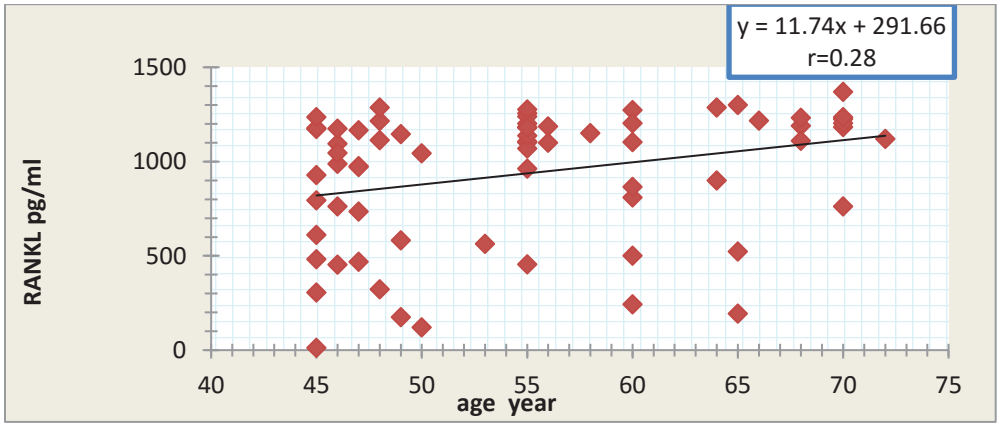

FIGURE 10. Correlation between RANKL level and age of osteoporosis in postmenopausal women.

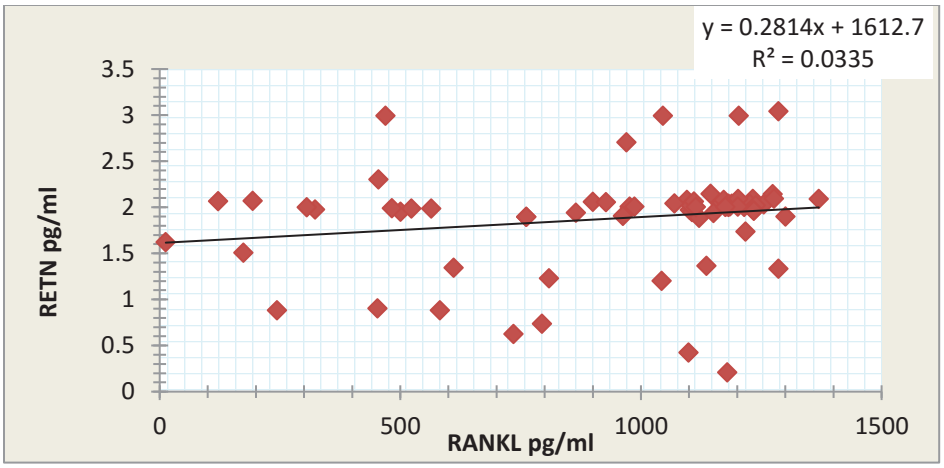

FIGURE 11. Correlation between RANKL level and RATN of osteoporosis in postmenopausal women.

DISCUSSION

The results of the study showed that the bone density data from a DEXA scan are reported as decrease in BMD and T-scores in menopausal women with osteoporosis when compared to healthy women. There were low significant differences in BMD between age groups. In this study show an age-dependent decline in BMD was seen in women in age groups and almost postmenopausal age were found to have low BMD of osteoporotic range; This finding is similar to studies [33,34].The study of [35] showed that the bone mass is a major determinant of bone strength and, after reaching peak values in the third decade of life, bone mass and density begins to decline until age 60-65, which result in low bone mass and decline of bone strength which is a risk factor for fragility fractures that occur with ageing and osteoporosis. The study revealed the osteoporosis most occur in the spin more than right femur when compared between the t-score in both sit. Also the study show the effect of BMI when compared between BMI groups that indicate the t-score in the normal weight less than over and obese weight, which because secretion of active bone hormones from pancreatic beta cell (insulin, amylin and preptin) and secretion of bone active hormones such as estrogen and leptin from adipocyte [36].

The results of this study have revealed a significant decrease ($P<0.05$) in serum estrogen in menopausal women with osteoporosis more than healthy women. This result agree with [37, 38]. In the menopause, estrogen levels rapidly decline, exposing women to a hypo-estrogenic state [39]. Thus, the exposure to estrogen through these key periods may dramatically influence a woman's bone health [40]. The estrogens are able to block bone resorption through two mechanisms: both by direct interaction with osteocytes and osteoclast and by regulation of T-cell and osteoblast formation and activity [41,42], therefore The decline of ovarian function at postmenopausal results in decreased production of estrogen; The combined effects of estrogen deprivation cause a marked stimulation of bone resorption and a period of rapid bone loss which is central for the onset of postmenopausal osteoporosis [43].

The result in the study show the significant decrease ($P<0.05$) in mean level of serum calcium and significant increase ($P<0.05$) in mean level of phosphorus, This finding was in accordance with result obtained in [44], which indicating that Decreased estrogen concentrations at menopause age lead to lower intestinal absorption calcium concentrations and increased osteoclastic resorption of bone. Both increase bone turnover and constitute risk factors for the development of osteoporosis. The factors that promote bone loss which is the calcium and vitamin D deficiencies through increase the bone resorption in order to maintain the blood calcium concentration [45,46].

The results of current study show a significant increase ($P<0.05$) in mean level of serum RANKL in osteoporosis group than healthy group, In postmenopausal women, hormonal changes cause an increase in receptor activator of nuclear factor Kappa-B ligand (RANKL), as does osteoclast activity; therefore leading to shift from bone remodeling toward bone resorption that leads to osteoporosis [47].

The remodeling of skeleton is maintain by the regulation coordination of bone-absorptive osteoclasts and bone-formative osteoblasts. Osteoclast differentiation is elaborately regulated by receptor activator of nuclear factor κB (RANK) expressed by osteoclast precursors, and receptor activator of nuclear factor κB ligand (RANKL), a cytokine mainly expressed by osteoblasts [48]. RANKL action contributes to tumor development [49], both postmenopausal

osteoporosis and tumor-associated bone loss can be effectively treated by RANKL antibody therapy [50]. The most of the bone diseases like postmenopausal osteoporosis had increased osteoclast activity [51].

The transcription factors NF-κB family is activated in response to a variety of stimuli, containing the TNF family of cytokines [52].This factors induced rapid transcription of genes cell survival, regulating inflammation, proliferation and differentiation [53]. NF- κB activation induced by RANKL results in a differentiating state of osteoclast precursors [54]; therefore in the study which found the level of RANKL in the group with osteoporosis more than in the group with osteopenia.

Receptor activator of nuclear factor k-B (RANK), its natural ligand to RANKL and osteoprotegerin (OPG) is the molecular link between oestrogen deficiency and bone loss [55]. RANK/RANKL/OPG axis also appears to play a key role in mediating the widely hypothesized connection between immune system and bone, central concept of osteoimmunology field [56].

In this study there was a significant increase ($P<0.05$) in mean level of serum resistin. [57]. The present study provided evidence that resistin, leptin IL-1, IL-4, IL-6 and TGF-β play important role in bone metabolism in postmenopausal women. These diagnostic markers may be able to identify patients at risk for osteoporosis. Resistin is an adipokine and induce the expression of cytokines and chemokines in human articular chondrocytes

Resistin play a role in bone remodeling [58]. The study have observed moderate correlations between resistin and a marker of increased osteoclast activity [59]. Resistin have important role in bone metabolism by stimulating osteoblast and osteoclast differentiation, possibly through the nuclear factor kappa B (NF-jB) pathway [60].

In the study that indicated present the negative correlation between the BMD and RANKL, this study that reported TNF-α is upregulated in postmenopausal women with osteoporosis and TNF-α could promote RNAKL-induced osteoclasts formation. It implied that TNF-α had a role in the development of postmenopausal osteoporosis dependent of the osteoclastic RNAKL/RANK pathway; the result of this study explain the present of negative correlation between the RANKL and BMD [54].

In postmenopausal osteoporosis, loss of estrogen leads to significantly increased receptor activator of nuclear factor B ligand RANKL expression. RANKL is a protein that is expressed by various cells including osteoblasts and bone lining cells and it is an essential mediator of osteoclast-induced bone loss [61, 62]. Increased levels of RANKL expression result in excess formation and activity of osteoclasts, leading to bone loss and fracture [63]; therefore. In this study found the negative correlation between the RANKL and E2.

CONCLUSION

This study present the positive correlation between the RANKL and RETN in menopausal women with osteoporosis, this indicate the role of inflammatory after menopausal and associated with bone resorpation.

SIGNIFICANCE STATEMENTS

This study is the first clinical study in Iraq.

ACKNOWLEDGMENT

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