Relevancy of blood specimen-molecular biomarkers in studying osteoporosis in postmenopausal women

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WORD COUNT	6209	TIME SUBMITTED	22-NOV-2024 11:55AM
		PAPER ID	113098163

Relevancy of blood specimen-molecular biomarkers in studying osteoporosis in postmenopausal women

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Abstract

Background: Osteoporosis is a bone disease with low bone mass and micro-architectural weakening of bone tissue. Osteoporosis can occur due in part to immunological activity, therapeutic targeting of the effector immune osteoporotic factors will protect an extended number of susceptible people especially postmenopausal women from this risky disease. The aim of the current study is to estimate blood levels of gene expression of RANKL, RANK, OPG, and OSCAR and the transcription factors $ROR_{\gamma}t$ and FOXP3 (as representative of Th17 and Treg cells respectively), and to determine their benefit in detecting postmenopausal osteoporosis cases.

Methods: A total of 88 post-menopausal women were included in the current study, 43 women with osteoporosis (PO group), 45 women without osteoporosis (PNO) as age-matched control group, in addition to 40 young healthy females as baseline controls (YA). Blood samples were collected from participants to estimate the expression levels of genes of interest by qRT-PCR.

Results: A non-significant elevation in RANKL expression in PO group compared to PNO and YA (PO=1.68, PNO=1.53 and YA=1.0). Th17 population is significantly elevated in PO group compared to YA (PO=1.53, YA=1.0). No statistical differences were found among the groups regarding other genes.

Conclusion: Th17 is a prominent osteoclastogenic marker detected in the blood. Additionally, RANKL which is crucial bone resorbing factor, however, detecting its expression level in the blood of postmenopausal osteoporotic women is almost inconclusive.

Keywords: postmenopausal women, osteoporosis, RANKL, RANK, OPG, OSCAR, RORγt, and FOXP3

INTRODUCTION

Osteoporosis is the most common bone disease characterized by low bone mass with weakening of microarchitectural bone tissue, accompanied by increased risk of fragility fractures [1]. Osteoporosis is a serious health problem; since it affects patients' quality of life, mainly due to femur and vertebrae fractures [2,3]. Due to increased ageing people in the population globally, osteoporosis incidence is growing rapidly and become a public health impact and is considered "the silent epidemic of the 21st century" [2]. Physiologically, bone undergoes a continuous regulated process of bone resorption and new bone formation called bone remodeling [4], which begins with the absorption of bone by osteoclast cells (OCL) and is followed by the generation of new bone by osteoblast cells (OBL). However, under physiological changes such as estrogen deficiency state in postmenopausal women or nutritional causes, bone resorption increases resulting in the primary type of osteoporosis. Osteoporosis can also occur as a consequence of certain diseases or drugs, a condition called secondary osteoporosis [5-7].

Although primary osteoporosis is classically regarded as an endocrine disease [8], many studies reported that the immune system is blamed for its pathogenesis. During the past two decades, a novel field called "osteoimmunology" was established to explore the relationship between bone homeostasis and immune systems [9]. OBL and OCL are activated in response to various mediators secreted from immune cells, including cytokines, chemokines, and growth factors [10].

OCL is the sole bone resorbing cells and is a key player in physiological bone remodeling and bone damage under pathological conditions such as osteoporosis, arthritis, periodontitis, and bone metastasis [11]. Both human bone marrow myeloid progenitor cells and circulating CD14+ monocytes in peripheral blood are considered sources of OCL precursor cells [12]. Under normal physiological conditions, OCL precursor recruitment, maturation, and function are under the fixed control of OBL. So, abnormality affecting OBL may cause dysfunction of OCL [13].

Once OCL precursor cells are recruited to the site of action, they will differentiate into mature functional multinucleated OCL cells [14]. Although it is well documented that various factors affect bone resorption, including hormones, cytokines, and noncoding RNAs, by mechanisms affecting signaling pathways in OCL differentiation. However, RANKL/RANK is the critical pathway among the other signaling pathways for osteoclastogenesis [15]. The co-presence of macrophage colony-stimulating factor (M-CSF) will upregulate this pathway. The binding of M-CSF with its specific receptor, colony stimulating factor 1 (c-Fms) on OCL precursors, activates intracellular signalling pathways resulting in upregulated expression of receptor activator of nuclear factor kappa-B (RANK) on these cells. RANK expression, in turn, will stimulate upregulated expression of receptor activator of nuclear factor-κB ligand (RANKL) by osteoblasts [16]. The subsequent binding of RANKL with RANK triggers the terminal differentiation into mature OCL [17, 18]. RANKL is a cytokine of tumor necrosis factor (TNF) family, physiologically produced by osteoblast and osteocytes, also by activated T cells and fibroblasts in inflammatory states [19, 20]. RANKL is indispensable for OCL differentiation; the loss or mutation of RANKL or its receptor RANK causes osteopetrosis [21, 22, 23]. To date, there is no clear evidence of RANKL-independent osteoclastogenesis or any factor that can replace RANKL functions, and RANKL is regarded as the sole master regulatory cytokine that specifically induces osteoclastogenesis [21].

The intracellular signaling pathway primed by RANK/RANKL binding is essential to OCL differentiation and activity [24]. RANKL/RANK binding initiates intracellular signals that recruit and activate tumor necrosis factor receptor-related factor-6 (TRAF-6) [25]. TRAF-6, in turn, activates NF-κB, which regulates osteoclast differentiation [26]. TRAF-6 also activates c-Src [27], which stimulates protein kinase B (PKB, Akt), which regulates osteoclast differentiation [28]. RANKL/RANK binding also activates the mitogen-activated protein kinase (MAPK) signaling pathway, resulting in the activation of transcription factors c-fos, activator protein-1 (AP1). AP1/c-Fos and NF-kB directly regulate the expression of nuclear factor of activated T cells 1 (NFATc1), the master transcription factor of OCL-specific genes [29, 30].

Although NF-kB and c-Fos/AP1 pathways have critical roles in initiating NFATc1 expression, they cannot sustain this expression [21]. RANKL is unique in having the potential to provide a persistent NFATc1 induction and auto-amplification [21]. RANKL achieve this activity by collaborating with signaling from co-stimulatory receptors, immunoreceptor tyrosine-based activation motif (ITAM), to initiate sustained calcium signalling which in turn induces

sustained NFTc1 expression [25,18]. OSCAR is one of ITAM co-stimulatory receptor family with a proven role in the regulation of osteoclast differentiation [31]. Blockade of OSCAR signaling was found to inhibit the formation of mature osteoclasts as well as their bone resorption activity [32].

For regulation of bone remodeling, OBL controls osteoclastogenesis by expressing a negative regulator of OCL differentiation which is osteoprotegerin (OPG), a secreted TNF receptor superfamily, which acts as a circulating decoy receptor for RANKL thereby preventing RANKL/RANK binding, resulting in suppressing OCL differentiation [33].

OPG is expressed in different tissues and circulates in the blood. Recent studies demonstrated that the OPG locally produced by osteoblasts, but not circulating OPG, is essential for bone homeostasis [34, 35]. Deletion of OPG in osteoblasts by using *Sp7*-Cre or *Dmp1*-Cre markedly decreased bone mass with no effect on serum OPG level, whereas OPG deletion in B cells (*Mb1*-Cre) or osteocytes (*Sost*-Cre) did not decrease bone mass [34,35].

Th17 cells have a primary role in the induction of various inflammatory conditions, such as osteoporosis, psoriasis, periodontal disease, rheumatoid arthritis and inflammatory bowel disease *via the* recruitment of immune cells [36, 37]. Retinoic acid-related orphan receptor-γt (RORγt) is a crucial transcription factor in Th17 cells providing the potential for pathological immune responses [38]. The differentiation and development of Th17 cells from naïve $CD4^+$ T cells require the presence of TGF-β, IL-6, IL-1β and IL-23 [39]. Th17 cells are often regarded as osteoclastogenic subsets of T lymphocytes [40], because they express high levels of RANKL, in addition, Th17 stimulates the expression of RANKL by osteoblasts and synovial fibroblasts *via* secretion of IL-1, IL-6, IL-17 and TNF [41, 42]. The enhancing role of Th17 in osteoporosis is supported by the increased Th17 cell population in the bone marrow, peripheral blood and the surrounding tissue of postmenopausal osteoporotic women [36]. Accordingly, estimating Th17 cell counts in the blood is an important marker for osteoporosis assessment [43].

Regulatory T cells (CD4+CD25+Foxp3+ T cells) are a special subset of T-helper cells that regulate the secretion and expression of pro-inflammatory and anti-inflammatory cytokines [36, 44,45]. The development and differentiation of naïve T cells to Tregs recommend the presence of IL-2 and TGF-β [46]. Functional Tregs should express Forkhead transcription factor (Foxp3) which has an important role in their development and function [44]. Tregs secrete cytotoxic T-lymphocyte antigen 4 (CTLA-4), which binds with CD80/ CD86 co-

receptors on OCL precursor cells inducing their apoptosis [47]. Also, Treg cells directly inhibit differentiation of OCL precursor cells by suppressing RANKL and M-CSF production *via* TGF-β and IL-10 production [18, 48]. It is well established that any dysregulation in the population or functioning of Tregs would result in enhanced bone loss [49]. Due to the plasticity of Treg and Th17 cells, Treg cells may lose their immunosuppressive function under estrogen deficiency and convert to Th17 cells, which explains the unbalance of Th17/Treg populations in postmenopausal osteoporosis [50].

METHODS

Study population

A case control study was conducted from December 2022 to August 2023 on 128 women who attended Rheumatology and Rehabilitation Clinic at Al Basrah Teaching Hospital: 88 of the studied women were post-menopause, and 40 were young cycling women. After obtaining their signed permission on a consent form, bone densitometry for the lumbar spines (L1-L4) done for each woman in the study to estimate Bone Mineral Density (BMD) and Trabecular Bone Score (TBS), expressed as T-score value for postmenopausal women and Z-score value for the young cycling females. According to WHO criteria, women with a T-score ≤-2.5 considered as postmenopausal osteoporotic group (PO) (n=43), women with T-score >-2.5 considered as postmenopausal non-osteoporotic group representing age-matched controls (PNO) (n=45), and young cycling females with Z-score >-2 as baseline control group (YA) (n=40).

Gene expression study

A total of 5 ml of blood samples were taken from each participant and placed in an EDTA tubes. RNA samples were extracted *via* GENEzol™ TriRNA Pure Kit (GZXD100, Geneaid, Taiwan). The cDNA was synthesized by EntiLink™ 1st Strand cDNA Synthesis Super Mix (EQ031-01, ELK biotechnology, China). The expression of RANKL, RANK, OPG, OSCAR, RORγt, and FOXP3 was estimated by qRT-PCR using EnTurbo™ SYBR Green PCR SuperMix (High ROX Premixed) (EQ013, ELK biotechnology, China). The primers used are demonstrated in table 1. The amplification program used was pre-denaturation stage at 95°C for 30 seconds, 1X, denaturation step at 95°C for 5 seconds, annealing step at 55°C for 30 seconds and extension step at 72°C for 30 seconds, the three steps were repeated for 39X, followed by the melting stage. The data was analyzed using the ΔΔCT method (Livak & Schmittgen, 2001).

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Statistical analysis

The data underwent statistical analysis using a normality test at the beginning followed by non-parametric test (Mann-Whitney U test) in the MINITAB program.

Table 1 Primers sequences for genes of interest and GAPDH used in qRT-PCR

Gene	Primer nucleotide sequences (5'-3')	Reference
RANK-F	5'-TTGCAGCTCAACAAGGACAC-3'	Primer design
RANK-R	5'-CGTAGGGACCACCTCCTACA-3'	Primer design
RANKL-F	5'-CCTGAGACTCCATGAAAACGC-3'	Primer design
RANKL-R	5'-TCGCTGGGCCACATCCAACCATGA-3'	Primer design
OPG-F	5'-CGGCGTGGTGCAAGCTGGAAC-3'	Primer design
OPG-R	5'-CCTCTTCACACAGGGTGACATC-3'	Primer design
OSCAR-F	5'-TGCTGGTAACGGATCAGCTCCCCAGA-3'	Primer design
OSCAR-R	5'-CCAAGGAGCCAGAACCTTCGAAACT-3'	Primer design
RORγt-F	5'-GCA GCG CTC CAA CAT CTT CT-3'	(Lin *et al*., 2015)
RORγt-R	5'-ACG TAC TGA ATG GCC TCG GT-3'	(Lin *et al*., 2015)
FOXP3-F	5'-CAC CTG GCT GGG AAA ATG G-3'	(Zhang *et al*., 2017)
FOXP3-R	5'-GGA GCC CTT GTC GGA TGA-3'	(Zhang *et al*., 2017)
GAPDH-F	5'-TGACCACAGTCCATGCCATCACTG-3'	Primer design
GAPDH-R	5'-CAGGAGACAACCTGGTCCTCAGTG-3'	Primer design

Results

The results of RANKL gene expression of the present study showed a non-significant elevation in RANKL expression in PO compared to PNO and YA, the expression values were PO=1.68, PNO=1.53 and YA=1.0. The p value for the three comparisons PO vs PNO, PO vs YA and PNO vs YA were 0.26, 0.09 and 0.43, respectively (Figure 1).

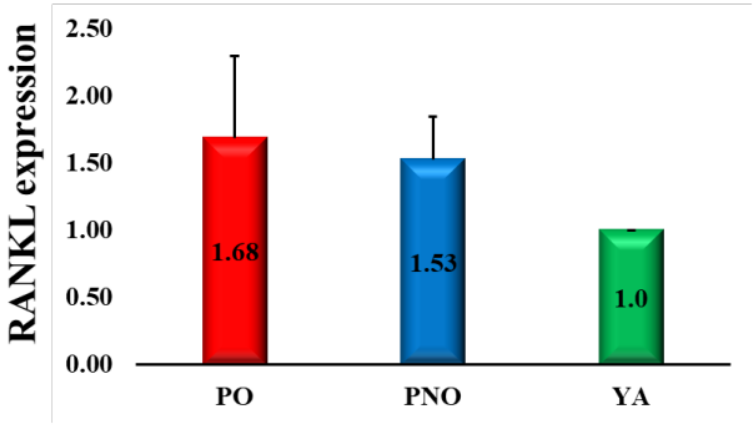

Figure 1 RANKL gene expression level in study groups.

The results of RANK gene expression of the present study showed no significant differences in RANK expression between PO and YA groups, the expression values were PO=1.03 and YA=1.0. RANK expression was non-significantly downregulated in PNO in comparison to PO and YA with a fold change of 0.85. The p value for the three comparisons PO vs PNO, PO vs YA and PNO vs YA were 0.38, 0.88 and 0.88, respectively (Figure 2).

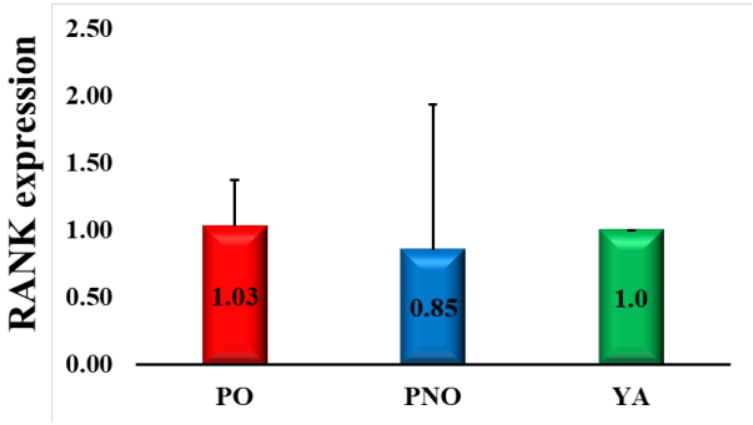

Figure 2 RANK gene expression level in study groups.

The results of OPG gene expression in the present study showed a non-significant difference in OPG expression among the study groups, the expression values were PO=1.26, PNO=1.33 and YA=1.0 . The p value for the three comparisons PO vs PNO, PO vs YA and PNO vs YA were 0.72, 0.29 and 0.43, respectively (Figure 3).

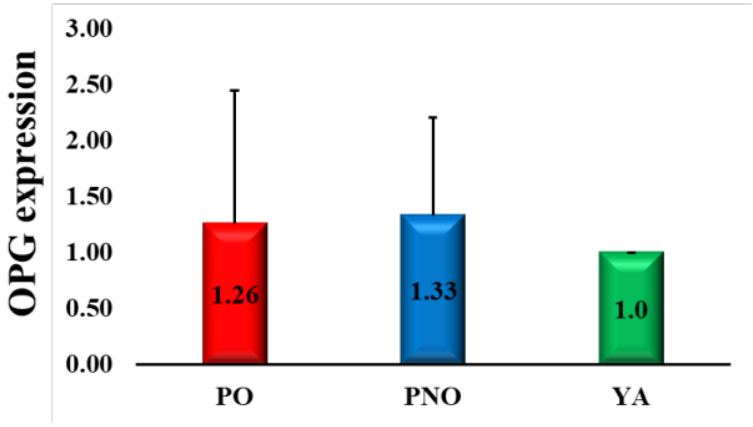

Figure 3 OPG gene expression level in study groups.

The results of OSCAR gene expression of the present study showed no significant differences in OSCAR expression between PO and YA groups, the expression values were PO=1.10 and YA=1.0 . OSCAR expression was non-significantly downregulated in PNO compared to PO and YA with a fold change of 0.74. The p value for the three comparisons PO vs PNO, PO vs YA and PNO vs YA were 0.38, 0.54 and 0.63, respectively (Figure 4).

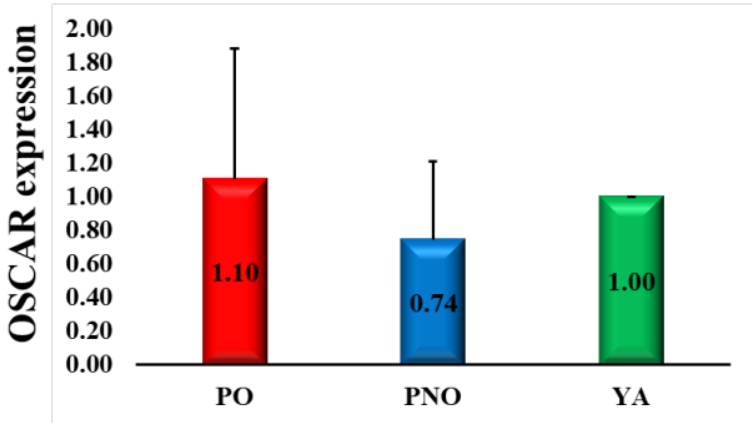

Figure 4 OSCAR gene expression level in study groups.

The results of RORγt gene expression of the present study showed a significant elevation in RORγt expression in PO compared to YA group, the expression values were PO=1.53 and YA=1.0, while RORγt expression in PNO showed non-significant downregulation compared to PO and no difference to YA (expression=1.07). The p value for the three comparisons PO vs PNO, PO vs YA and PNO vs YA were 0.4, 0.02 and 0.88, respectively (Figure 5).

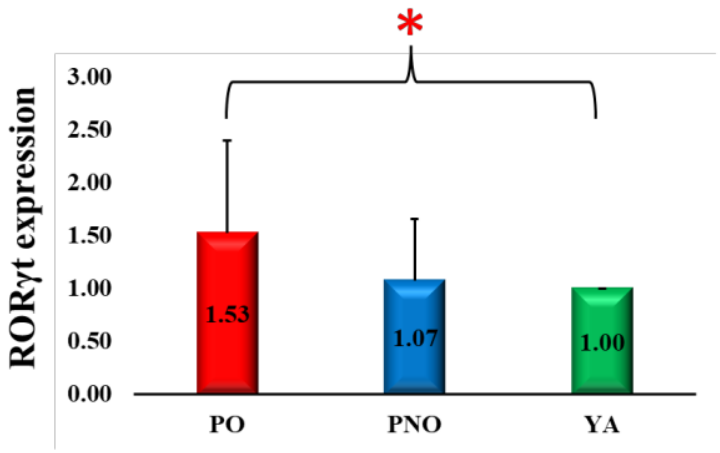

Figure 5 RORγt gene expression in study groups.

The results of FOXP3 gene expression in the present study showed a non-significant difference in FOXP3 expression among the study groups, the expression values were PO=1.06, PNO=0.95 and YA=1.0. The p value for the three comparisons PO vs PNO, PO vs YA and PNO vs YA were 0.92, 0.65 and 0.88, respectively (Figure 6).

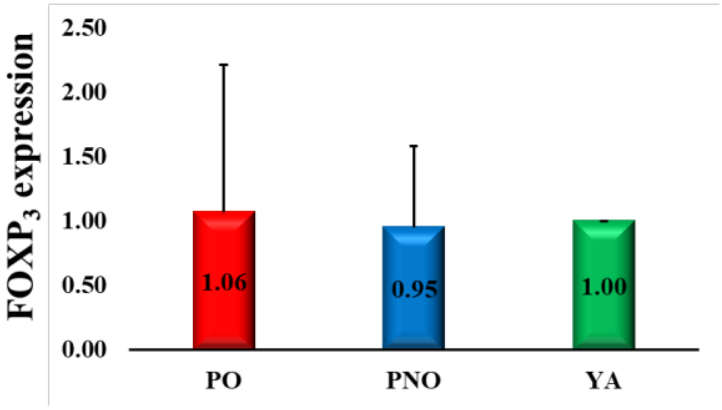

Figure 6 FOXP$_3$ gene expression in study groups

DISCUSSION

1-RANKL expression

Apart from the PO group having the highest expression level, the current study showed no significant difference in RANKL gene expression among the study groups. This result may be attributed to the well-documented finding that osteoblasts and osteocytes are the main sources of RANKL [19,20]. Therefore, in the current study, the estimated blood levels of RANKL gene expression are expected mainly from activated T and B lymphocytes that can produce RANKL in response to pathological conditions such as inflammatory states [19, 20]. This suggested explanation may be supported by detecting the highest RANKL expression level in PO group since osteoporosis is regarded as an inflammatory condition [60]. At the same time, this study finding indicates that RANKL produced by these inflammatory cells is insufficient to reach significant levels, this assumption is supported by the reported finding of variations in the results between peripheral circulation products and the local expression level of bone metabolism markers [53]. Further, this result indicates that the accurate estimation of RANKL gene expression needs to work on bone cells directly. On the other hand, the finding of no statistical difference in RANKL expression may be due to possible presence of RANKL gene mutations in PO group population that cannot be detected in the current study, further study searching for RANKL gene mutations that might be detected in circulating RANKL producing cells may be required to confirm this proposed explanation.

2-RANK expression

The current study shows no significant difference in the level of RANK expression among the three groups. This finding may indicate that RANK-expressing cells that circulate in the blood which are mainly monocytes and dendritic cells (apart from myeloid progenitor cells in bone marrow) do not over-expressed RANK gene unless they are recruited to the bone surface area and then stimulated to become osteoclast precursor cell and upregulate RANK expression, this explanation is supported by the reported finding that direct local contact on the bone surface between osteoblast and osteoclast precursor cells (including resident macrophages, recruited monocytes, dendritic cells and the marrow myeloid precursor cells) is needed to initiate upregulation in RANK gene expression on osteoclast precursor cells [13, 17, 18].

3-OSTEOPROTEGERIN expression

The current study results showed no statistical difference in the levels of OPG expression among the study groups. A possible explanation for this result is that OPG gene expression detected in the peripheral blood is from circulating leucocytes and this product has no effect on bone homeostasis, at the same time, this may indicate that the OPG produced locally by osteoblasts is the main regulator of bone mass *via* its function as a decoy receptor for RANKL, preventing RANKL/RANK binding. This explanation is supported by the previously documented finding that locally produced OPG by osteoblast is essential for bone homeostasis while circulating OPG do not affect bone homeostasis [34,35]. The current study finding is further supported by the reported studies that find deletion of genes responsible for OPG expression in osteoblast leads to a marked decrease in bone mass with no effect on OPG serum level, on the contrary, deletion of OPG genes in B lymphocytes and osteocytes leads to a decrease in OPG serum level with no effect on bone mass [34,35].

4-OSCAR expression

RANKL has a unique ability to generate sustained activated calcium signaling in OCL precursor which causes persistent activation of NFATc1. Sustained stimulation of NFATc1 is mandatory to complete the osteoclastogenesis process [21]. RANKL performs this task by collaboration with signals from ITAM containing immunoglobulin-like co-receptors. OSCAR is one of these co-receptors with known potential to co-stimulate persistent calcium signaling [11], the role of other ITAM co-receptors in osteoclastogenesis is not well understood [31]. OSCAR in humans is expressed by OCLs, also by monocytes, macrophages and dendritic cells [54]. During bone remodeling, the recruitment of OCL precursor cells from the circulation to bone surfaces requires trans-endothelial migration through blood capillaries that express RANKL and collagen III, subsequently, these cells may contact osteoblast cells on the bone surface or be exposed to collagens I and III from the bone matrix [55]. Interaction between OSCAR on OCL precursor cells and these collagen ligands in the presence of RANKL may facilitate the production of multinucleated OCLs [55]. OSCAR role in bone lesions of Rheumatoid arthritis patients is well confirmed. Rheumatoid arthritis (RA) is an autoimmune disease with a tendency to cause inflammation of the synovial membranes with infiltration of inflammatory cells and increased production of activated OCLs, which induce bone erosion and joint destruction [56]. Joints of RA patients show mature OCLs close to the bone surface, with numerous mononuclear OCL precursors at different stages of maturation

found in the surrounding joint tissues, these cells are attracted from peripheral blood through synovial microvasculature , OSCAR has been detected in all these cells [57, 58]. Herman's study showed that OSCAR is highly expressed by multinucleated OCLs at the bone surface and approximately 30% of mononuclear cells around synovial microvessels. In addition, circulating monocytes from peripheral blood of RA patients showed 2-fold higher expression of OSCAR than monocytes from healthy individuals, suggesting that the receptor expression is increased before the cells enter the joint compartment [58]. The current study shows no significant difference in OSCAR expression among the study groups, this study result disagreed with Herman *et al*.(2008). The possible explanation for such disagreement with Herman *et al*, (2008) may be attributed to the effect of abundant proinflammatory cytokines in patients with active RA, where it is documented that TNFα is the main inducer of OSCAR expression in monocytes [58], this explanation is further supported by the reported finding that levels of OSCAR are higher in the synovial tissue of active RA compared to inactive RA due to the effect of abundant proinflammatory cytokine in the active state of RA [59]. Accordingly, the finding of no significant difference in OSCAR expression among the study groups may indirectly lead to the conclusion that if postmenopausal osteoporosis is regarded as an inflammatory process [60], then it seems to be of chronic low-grade type with no abundance of proinflammatory cytokines. As far as the researcher's knowledge, no similar study investigating the level of OSCAR expression in the peripheral blood of post-menopausal osteoporotic women was found to compare.

5-ROR$_\gamma$T expression:

Studies indicated that Th17 cells play an essential role in bone remodeling by promoting osteoclastogenesis [47]. Th17 cells can induce bone loss directly through expressing RANKL [49]. Furthermore, Th17 cells enhance the expression of RANKL by osteoblasts and fibroblasts *via* the production of inflammatory cytokines, such as IL-6, IL-17 and TNF-α, and subsequently promote bone resorption [61]. The key transcription factor (representing the signature defining transcription factor) of Th17 cells, is retinoic acid receptor-related orphan nuclear receptor gamma (ROR$_\gamma$t), which is essential and sufficient to induce Th17 cell linage in humans [62, 63]. Accordingly, the expression level of ROR$_\gamma$t was investigated in the current study as a representative of Th17 cell population in the peripheral blood of the participants. ROR$_\gamma$t expression in PO group showed statistically significant elevation compared to YA group, while no significant difference was found between PNO and YA

group. This finding agrees with the documented observation that elevated Th17 cell population is associated with low bone mineral density [64]. In addition, many clinical studies have shown that the frequency of Th17 cells in the circulation and bone surrounding tissues of osteoporosis patients is higher than that in persons free of osteoporosis [49]. Thus, this study may indicate that Th17 cell regulation may be an important target in osteoporosis suppression.

6-FOXP3 expression

Foxp3, a member of the forkhead box family of transcription factors, is currently regarded as a specific identification marker of Treg cells and is also an essential molecule for the development and functional expression of Treg cells [65]. Identifying Foxp3 as the Treg cells representative factor is sufficient for specific in vivo targeting of Foxp3+ Treg cells [66]. Studies have shown that the Treg cells affect bone by two mechanisms: direct cell contact and the release of anti-inflammatory cytokine [67]. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) is a surface protein on Treg involved in contact inhibition of osteoclast formation [68]. Treg cells expressing CTLA-4 bind to CD80/CD86 on the surface of osteoclast precursor cells, this binding results in the activation of indoleamine-2,3-dioxygenase in osteoclast precursor cells, this enzyme can degrade tryptophan, promote the apoptosis of osteoclast precursor cells [69]. Treg can further inhibit the production of osteoclasts by preventing the production of RANKL and M-CSF, by releasing the inhibitory cytokines IL-10 and TGF-β, leading to an increase in bone mass [18]. No consensus regarding the inhibitory mechanism of Treg cells on osteoclastogenesis has been established [70]. Kim *et al*. found that Treg cells inhibit osteoclast differentiation from precursor cells in a cytokine-dependent manner and there is no rule for cell to cell contact mechanism, they proposed that TGF-β and IL-4 may be the essential cytokines for the suppressive function of Treg cells [71]. In contrast, Zaiss *et al*. concluded that the predominant mechanism of Treg cells induced osteoclastogenesis suppression is by cell to cell contact by CTLA-4, suggesting that IL-4 and IL-10 contributed to, but were not necessary for the inhibitory effect [67]. In the current study, no significant differences in the expression level of FOXP3 were found among PO, PNO and YA groups. There are two possible explanations for this result, the first suggested explanation depends on Zaiss *et al*.(2007) conclusion, therefore, Treg cell population is expected to be more abundant near the bone surface area to come in contact, through CTLA-4, with osteoclast precursor cells inducing apoptosis of these precursor cells

to suppress the inflammatory process, since osteoporosis regarded as a chronic inflammatory condition [60]. According to this explanation, the estimated expression level of FOXP3 in peripheral blood cannot give the true estimate of Treg population because these cells are more abundant near the bone surface to perform their task. The second possible explanation depends on Kim *et al*.(2007) conclusion already mentioned, which suggests that Treg suppress osteoclastogenesis through cytokines and not through cell to cell contact, the second explanation may be supported by finding the expression level of $ROR_{\gamma}T$ is proportionally higher than the expression level of FOXP3 (1.526 and 1.064 respectively) in PO group, which mean presence of higher population of Th17 than Treg in PO group (a state of dysregulated balance between Th17 and Treg), resulting in elevated proinflammatory cytokines and suppressed anti-inflammatory cytokines that leads to rapid bone loss. In contrast, the PNO group showed almost no difference in the expression level of these two transcription factors ($ROR_{\gamma}T$: 1.072 and FOXP3: 0.953), indicating that the physiological balance between Th17 and T-reg cells still existed and regulate osteoclastogenesis in this group.

CONCLUSION

Estimating gene expression levels of immune markers correlated with postmenopausal osteoporosis including RANKL, RANK, OPG, OSCAR in peripheral circulation is almost inconclusive in detecting postmenopausal osteoporosis cases. Estimating Th17 population in peripheral blood and searching for dysregulation in the balance between Th17 and Treg in such samples is beneficial in tracing expected postmenopausal osteoporotic cases.

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