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Serum Levels of Receptor Activator of Nuclear Factor-κβ Ligand, Osteoprotegerin, Interlukin-17 and association with Receptor Activator of Nuclear Factor Kappa B/ Osteoprotegerin Ratio in Patients with Osteoporosis.

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Abstract:
An imbalance between the formation and resorption of boneleading to osteoporosis disease which described as drops level of bone mineralization that result in bone fracture.The current case control study determined levels of (RANKL), (OPG) and IL-17 in serum and the RANKL/OPG ratio for detection this disease , in addition to detect IL-17 level in patents serum. Method: fifty patientswith osteoporosis from both genderwith the age range between (50-88)represented (group1)and (group 2) of 40 healthy control personsdetection of these biomarkersdone by ELISA test. The OPG/RANKL ratio was also calculated. In addition to detection the role of IL-17 in osteoporosis. RANKLlevelsin patients more than those of control, the difference was high significant as $P < 0.001$, serum levels of OPG were lower in patients group in comparison with control group and the difference was highly significant ($P < 0.001$).The ratio of RANKL/OPG in patients group was $P<0.001$(raised significant) compared to the that of healthy subjects. Serum levels of IL-17in patients were raising morethan in control with ($P < 0.001$).
Conclusions: Serum levels of RANKL, OPG and RANKL to OPG percentage were potentialbiomarkers for detection osteoporosis, also, serum IL-17 was significantly elevated in osteoporotic patients when compared to healthy control both in male and female patients with osteoporosis.

Introduction:

Osteoporosis is a skeletal condition defined by a decline in bone mineral density (BMD) and mass resulting in impaired bone structure, when the body loses more bone and /orproducesfew bone,diminished density can occur, as a result, thereducedbone strength clinically appeared as bonefracture [1].
In Iraq, osteoporosis is regarded as a main health problem.Iraq's current population is projected to be 29.6 million people, with 9.5 percent (2.8 million) over the age of 50 and 1.9 percent (570000) over the age of 70. It is expected that by2050, 26% (15 million) of the population will be 50 years old or older and 7.2 percent (4 million) will be 70 years old or older, with a total population of about 56 million.[2].Varied cytokines, proteases, and morphogens have been reported to act a key role in bone remodeling[3, 4]. Molecules such asRANKL,RANKandOPGare among bone biochemical markers that depicts the metabolic states of osteoblasts and osteoclasts [5,6].
RANKL-RANK interaction form RANKL/RANK system which trigger resorption events to

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started[7].The decoy receptor for RANKL,is OPG (TNFRSF11B) which is fundamentally released by osteoblasts. Competition of OPG with RANK and antagonize the effects of RANKL-RANK reaction inhibit osteoclast differentiation and activation[8].

Osteocytes form the main structures of cortical and calcaneus bone and have multiple physiological function for bone resorption or formation. Osteocytes encourage the production of RANKL and lowered OPG expression. As a result, RANKL-OPG ratio raises, osteoclastogenesis happens and the improvementof bone resorption in the unloading activity is appeared[9].The serum RANKL-OPG ratio is a critical agentto determineactivationof osteoclast [10].

In addition to RANKL and OPG, both adaptive and innate immune cells (T cells, B cells, macrophages and dendritic cells) produce significant amounts of pro-inflammatory cytokinessuch as IL-17lead to osteoporosis downstream of inflammatory disease [11,12].

One of T lymphocytes subsets, Th17, which marked as osteoclastogenic cells[13, 14]. Osteoclastgenesis induced by Th17 high secretionsof cytokines (IL-1, IL-17, IL-6, TNF and RANKL)but low levels of IFN-γ [15, 16] also, osteoclastogenesis improved by action of IL-17 which induce osteoblasts, osteocytes and strengthen osteoclastogenic activity by upregulation of 'RANK' production and great production of RANKL[17,18].

Therefore this study is aimed to measure the circularity levels of RANKL, OPG and IL-17 during osteoporosis both in male and female patients, in addition, the current research also aimed to detect RANKL/OPG ratio.

Methods:

The current research was performed on fifty patients suffering from osteoporosis according to physician diagnosis in Rheumatology Consultation Clinic of Marjan Teaching Hospital (Babylon province, Iraq) patients' age range between 50-88 yearsfrom March to August 2020.. Other groups consist of 45 healthy individuals with an age range between 55-87 years without any history of systemic disease were clinically considered as healthy also included in this study as a control group. We excluded patients with renal failure, patients with cancer, kidney failure and patients undergoing treatment for osteoporosis.Blood sample were collected by venipuncture from these groups (three ml of venous blood) were drawing by disposable syringe into sterile plain tube under aseptic technique then and allow sample to clot for a few minutes at room temperature then followed by separation of serum from the clot by centrifugation for 10 minutes at 2500 r.p.m. then serum transferred to an Eppendorf tube labeled and stored at -20℃ until use for ELISA assay to avoid repeated thawing and freezing.This study was in agreement with ethics of of Marjan Teaching Hospital and verbal informed consent was obtained from all participants.
ELISAkit provided from Bioassay Technology, (China) was used to determine the level of three biomarkers in human serum including : Cat.No E0620Hu for Human Receptor Activator of Nuclear Factor Kappa B Ligand(RANKL), Cat.No E1558Hu for human Osteoprotegerin (OPG) and Cat.No E0142Hu for Human Interleukin 17 Assays, all of them were done according to manufacturer’s manual.

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Statistics analysis :were summarized, presented and analyzed using statistical package for social science (SPSS version 24). Numeric data were presented as mean, standard deviation, median and interquartile range (IQR) but nominal data were articulatedin number withpercent values. Particular sample T test was performed to assess mean of two parametric groups , whereas "Mann Whitney U" test was applied to analyzemedian values betweenboth groups of nonparametric while nominal statistics was inspected by employing Chi-square . Correlation cofficiant was estimated by spearman correlation.

1-Characteristics of patients and control subjects

This research enrolled fifty patients (50) with Osteoporosis and forty (40) apparently healthy subjects. The demographic characteristics of patients and control subjects are shown in table (1). The mean age of patients was 72.5 ± 9.45and that of control subjects was 71.4 ± 8.33 years and the results showed no significant difference between patients and control groups in mean age (p= 0.561). Again, the results yielded no significant differencein patients and control groups in the distribution of frequency according to age (p= 0.699). Patients' group included 8 (16 %) males and 42 (84 %) females, whereas, control group included 10 (25 %) males and 30 (75 %) females.According to gender, no significance in difference of the frequency distribution in patients and control (p= 0.289).

According to residency, patients' group included 30 (60 %) cases from urban areas and 20 (40 %) cases from rural areas, while control group included 28 (70 %) cases form urban areas and 12 (30 %) cases from rural areas, in term of residency, no significant difference in patients compared to control persons (p= 0.325).According to BMI, the mean of BMI of patients was 30.07 ± 5.1and that of healthy control subjects was 30.55 ± 4.07 and also no significance in the differences among patients and control in mean BMI (p= 0.628). The above results have ensured statistical matching between patients' group and control group regarding age, gender and residency which is a prerequisite for such case control study.

[Table 1]: Demographic characteristics of Osteoporotic patients and control subjects

Characteristic	Patients Group	Control Group	p
Age [years]			
Mean ±SD	72.5 ± 9.45	71.4 ± 8.33	**0.561 † NS**
Range	50-88	55-87	
< 65, *n* (%)	11 (22 %)	6 (15 %)	**0.699 ¥ NS**
65-75, *n* (%)	18 (36 %)	16 (40 %)	
≥ 75, *n* (%)	21 (42%)	18 (45 %)	
Gender			
Male, *n* (%)	8 (16 %)	10 (25 %)	**0.289 ¥ NS**
Female, *n* (%)	42 (84 %)	30 (75%)	

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Male: Female	1:5.25	…….	
Residency			
Urban, *n* (%)	30 (60 %)	28 (70%)	**0.325 ¥ NS**
Rural, *n* (%)	20 (40 %)	12 (30 %)	
BMI			
Mean ±SD	30.07 ± 5.1	30.55 ± 4.07	**0.628 † NS**
Range	18.20-38.80	22.0-36.0	

n: cases' number , †: independent 'samples'of t-test , **SD**: standard of deviation, ¥: 'Chi_square' test, **NS**: not' significant' at level of P> 0.05, HS: highly significant at P ≤ 0.05.

2- Risk Factors of Osteoporosis patients.

To study a possible effects of risk factors such as (body mass index, history of previous Fracture, and smoking on the Osteoporosis disease, table (2)appear that there was higher percentage of Osteoporosis patient associated with body mass index, where 29 (58%) of Osteoporosis patients were obese, although the relation was not significant when compared to the healthy controls (P = 0.170).

The frequency distribution of Osteoporosis patients and healthy controls according to History of previous fracture was as following: 20 (40 %) patients having previous fracture and 30 (60 %) don't having previous fracture in compared to healthy controls was 0(0 %) with previous fracture and 40 (100.0 %) without previous fracture, (table 5). These result indicated the prevalence of osteoporosis washigher among patients who have history of previous fracture (P < 0.001).

According to some studies, more than 50% of postmenopausal women were suffering from osteoporotic fracture and were expected to increase along with the life expectancy. The burden of osteoporosis on family or society is high due to its high prevalence and the serious consequences of osteoporotic fracture [19].

[Table 2]: Frequency Distribution of Patient withOsteoporosis According to Some Risk Factors.

Characteristic	**Patients**	**Control**	***P***
BMI			
Underweight (< 18.5) , *n* (%)	3 (6%)	0 (0%)	**0.170 ¥ NS**
Normal (18.5- 24.9), n (%)	4 (8%)	4 (10%)	
Over weight (25 - 29.9), *n* (%)	14 (28%)	18 (45%)	
Obese (> 30), *n* (%)	29 (58%)	18 (45%)	
History of previous Fracture			

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Yes, *n* (%)	20 (40 %)	0 (0 %)	**< 0.001 ¥ NS**
No, *n* (%)	30 (60 %)	40 (100%)	

n: number of cases; ¥: Chi-square test; **NS**: not significant at *P*> 0.05**; S**: significant at *P* ≤ 0.05

3-Subjects Immunological Analysis Results

3-1- Serum RANKL level in patients and control groups.

The comparison of serum RANKL level between patients and control groups has been carried out and the results were demonstrated in table (3) and figure (1), Median levels (IQR) of serum OPG were 106.11(265.94) ng/L and 36.03(19.07) ng/L, in Osteoporosis patients and control group respectively; the level was higher in patients group in comparison with control group and the difference was highly significant (P < 0.001).

These results are in agreement with those ofAl-Masaoodi *et al*,2019 [20]which conducted in Iraq, this research showed a significant increase (P<0.05) in mean level of serum RANKL in osteoporosis group(postmenopausal women) 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 [21].

However, in our knowledge, there are few studies on osteoporosis in men to compare our results with it and osteoporosis in menstay a poorly studied medical problem despite its significance. It is estimated that at least 1 of 5 men will suffer from osteoporotic consequences. Osteoporosis deals with patients' age indicated significant highest in serum levels of bone specific alkaline phosphatase,phosphate, TNF-α, IL-6, IL-1β, CRP, NO, MDA and gene of RANKL expression, there was significant lowering inthe serum level of OPG[22].

Villiers, 2015 [23] reported that estrogen deficiency in osteoporosis patients causes an increase in active osteoclasts with increased bone resorption and loss of bone mineral density may be by effect in cytokines and the receptor activator of nuclear factor (RANKL) system.

Different cytokines that bind to their receptors in osteoblasts which hypothesized by Lorenzo., *et al* 2017 [24] to result in releasing of soluble factors act directly on osteoclasts to modulate recruitment and inhibit releasingthe stimulatory factors from osteoclast or could enhance releasinginhibitory factors from osteoclast .

In addition, many cytokines alter proteins of osteocyte signaling, osteocyte-to-osteoclast signaling is enhanced largely by multiple proinflammatory cytokines by RANKL signaling[25].

[Table 3]: Comparison of serum RANKL levels between healthy controls and patients with Osteoporosis.

	Case – control comparison		
RANKL (ng/L)	**Patients n = 50**	**Control n = 40**	***P***
Range	23.93– 566.53	20.0 – 428.09	**< 0.001 †**

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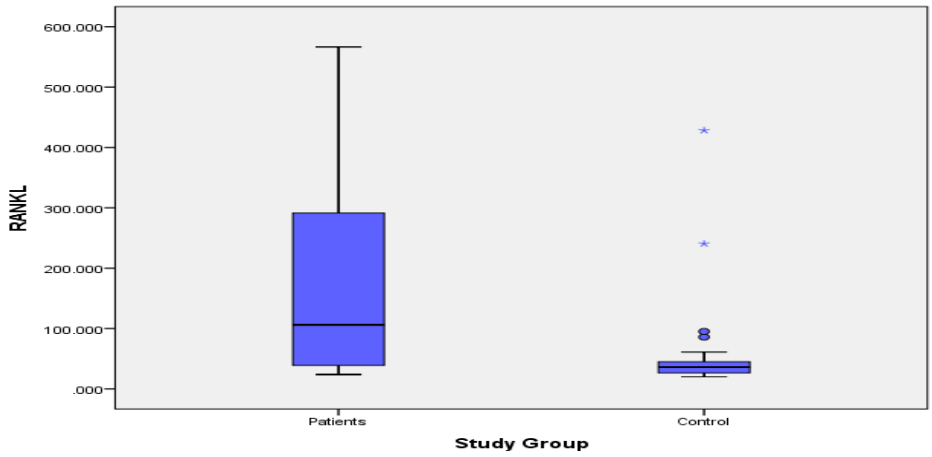
Median (IQR)	106.11(265.94)	36.03(19.07)	**HS**
Mean Rank	56.38	31.90	

n: for cases'number,†: for Mann Whitney U test,IQR: inter quartile range,HS: Highly significant at $P \leq 0.001$



[Figure 1]: Distribution of osteoporotic patients and control groupsat level of serumRANKL.

Serum RANKL level was significant correlated to age, gender or History of previous Fracture (P < 0.05), however, serum RANKL level was not significant correlated to BMIand family history of patients (P > 0.05), as shown in table (4).

Abdallah *et.al.*[26]observed elevated mRNA rate ofRANKL to OPG in women by testing bone biopsies with hip fractures.

[Table 4]: Correlations of serum RANKL according to Age, Gender, BMI, active smoking,History of previous Fracture, Family history of patients with Osteoporosis

Characteristics	**Patients groups**	
	R	**P**
Age	-0.226	0.032 S
Gender	0.244	0.021 S
BMI	0.068	0.526 NS
Family history	-0.069	0.369 NS

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History of previous Fracture	-0.221	0.036 S

r: Spearman correlation coefficient; HS: highly significance at $p \leq 0.001$, NS: non significance at $p > 0.05$.

3-2- Serum OPG level in patients and control groups.

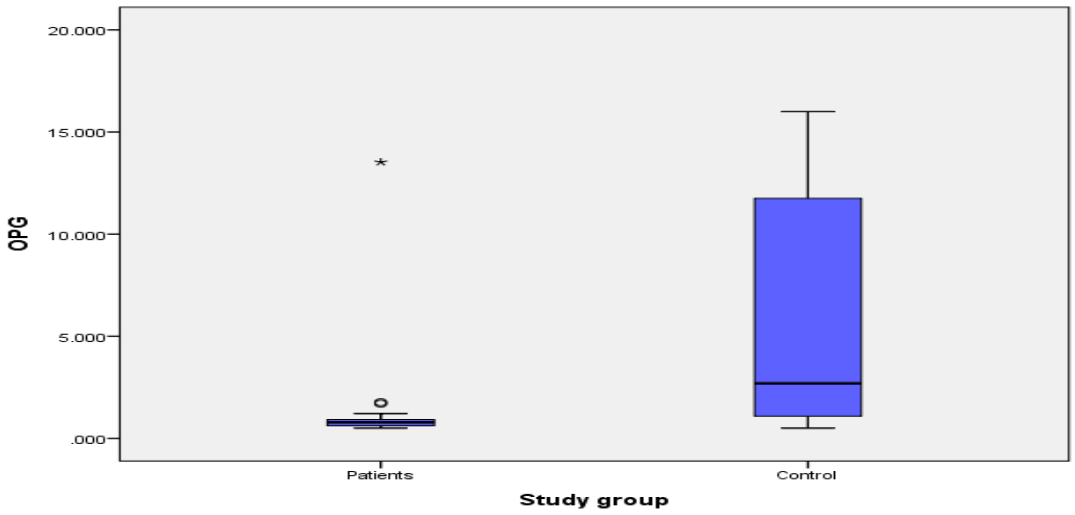
The comparison of serum OPG level between patients and control groups has been carried out and the results were demonstrated in table (5) and figure (2), median levels (IQR) of serum OPG were 0.78 (0.3) ng/L and 2.69 (10.73) ng/L, in osteoporosis patients and control group respectively, in relative to the control group, the amount was lesser in the patients' groupand the change was highly valuable (P < 0.001).

[Table 5]: Comparison of serum OPG levels between healthy controls and patients with Osteoporosis.

	Case – control comparison		
OPG (ng/L)	**Patients n = 50**	**Control n = 40**	***P***
Range	0.5– 13.52	0.5– 16.00	**< 0.001 † HS**
Median (IQR)	0.78 (0.3)	2.69 (10.73)	
Mean Rank	29.38	65.65	

n: number of cases, IQR: inter-quartile range,†: Mann Whitney U test, HS: Highly significance at $p \leq 0.001$

By functioning as a RANKL decoy receptor, OPG created by osteoblast cells adjusts the maturation and activity of osteoclasts.As a consequence, each strength and mass of bone are influenced by proportional concentrations ofRANKL and OPG[27].



[Figure 2]: Distribution of osteoporotic patients and control group inOPG levelsinserum.

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Serum OPG level was not significantly correlated to age, BMI, or active smoking of patients (P > 0.05), however, serum OPG level was significantly correlated to gender,history of previous fracture and family history of patients (P < 0.05), as in table (6).

[Table 6]: Correlations of serum OPG according to Age, Gender, BMI, active smoking,History of previous Fracture, Family history of patients with Osteoporosis

Characteristics	Patients' group	
	R	P
Age	-0.146	0.169 (S)
Gender	-0.209	0.048 (S)
BMI	-0.108	0.310 (NS)
Family history	0.379	0.001(HS)
History of previous Fracture	0.342	0.001 (HS)

r: coefficient of Spearman correlation; NS : non-significance at *p*> 0.05, HS: Highly significance at $p \leq 0.001$

In other research, comparing with those of normal BMD,certain subjectshad drastically reduced median serum levels of OPG than subjects with normal BMD (P=0.004), even though the two groups had similar levels of RANKL, RANKL- OPG correlation was greater in women with low BMD (P =0.027) [28]. Same study proved that age and years after menopause were found to influence levels of bone markers consisting OPG and RANKL- OPG proportion in serum, suggesting that these are confounding factors.BMI, on the other hand, had no effect on the same variables,OPG gene depletion in human and rat genomic DNA can create massive osteoporosis [29].

3-3- Interleukin-17 levelsin Serum:

Serum IL-17 levelsin patients and in control persons has been carried out and the results were demonstrated in table (7) and figure (3), median levels (IQR) of serum IL-17 were 81.8 (363.53) ng/L and 33.21 (19.33) ng/L, in osteoporosis patients and in control group, theselevels were elevated in patients group than in control group with large significancethat p lower than 0.001.

Our results in agreement to other results performed in Iraq on postmenopausal women which showed that the mean of serum IL-17 was (0.497pg/ml) and it is significantly higher than that of healthy group (0.096pg/ml)[30].Prior findingshad recorded elevatedcytokinesfrom Th17 in serumof women hadosteoporosis disease [31].

Comprehensive analysis of bone defects revealed thatTh17 cells and their proinflammatory IL-17cytokine facilitate bone degradation and are accountable inosteoporosis of estrogen deficiency [32].

In addition to that,IL-6,IL-17, IFN-γ and TNF-α encourage osteoclastogenesis

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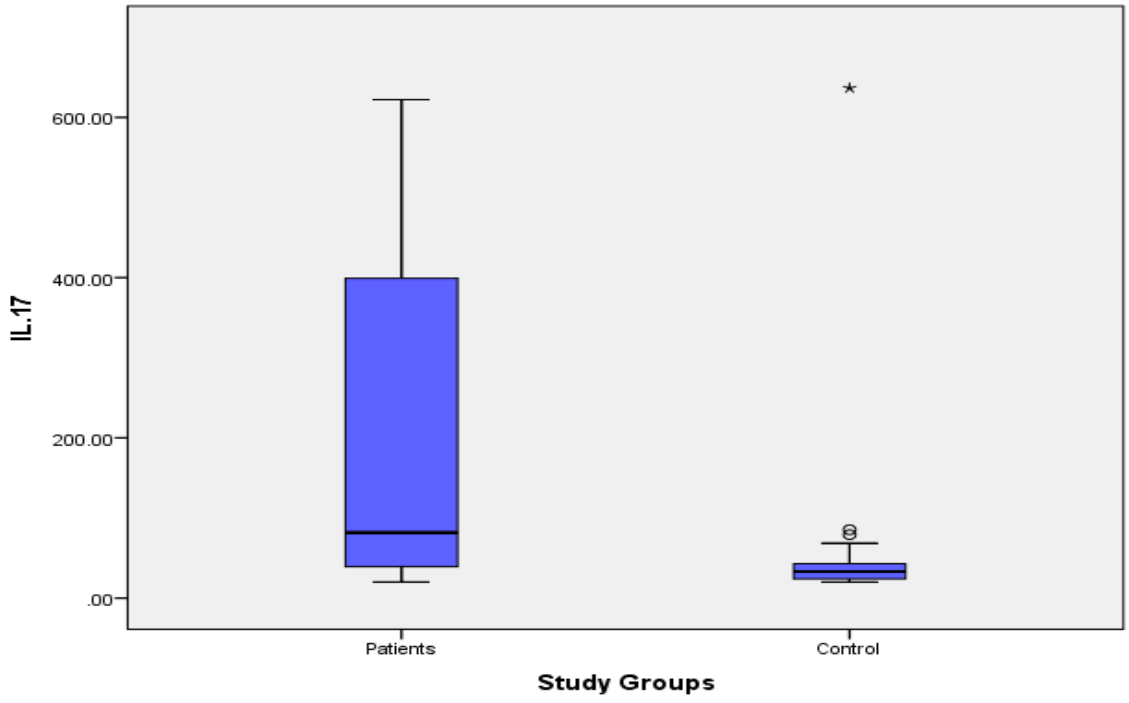
whiledisrupting differentiation of osteoblast which outcomein extremelyreduction in the density of bone structure[33].

IL-17 can play in inducing chronic inflammatory events such as bone loss[34,35,36,37], it wasproved thatbone lossencouraged by IL-17 via enhancing osteoclast formation and hindering differentiation of osteoblast cells [38].

[Table 7]: Differences of RANKL-OPG proportion in patients and controls

	Case – control comparison		
IL-17(ng/L)	**Patients n = 50**	**Control n = 40**	***P***
Range	20.00– 622.13	20.00 – 636.66	**< 0.001 † HS**
Median (IQR)	81.8 (363.53)	33.21 (19.33)	
Mean Rank	57.56	30.43	

n: number of cases,†: 'Mann Whitney U ' test,IQR: inter-quartile range, HS: Highly significance at $p \leq 0.001$.



[Figure 3]: Arrangement of osteoporotic patients and control group based on the Serum Interleukin-17.

Serum IL-17 level was significantly correlated to age, gender or history of previous fracture of patients (P < 0.05), however, serum IL-17 level was not significantly correlated to BMI,smoking and family history of patients (p > 0.05),table (8).

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[Table 8]: Correlations Interleukin-17 according to Age, Gender,BMI, active smoking,History of previous Fracture, Family history of patients with Osteoporosis

Characteristics	Patients groups	
	R	P
Age	-0.250	0.017 (S)
Gender	0.354	0.001(HS)
BMI	0.61	0.570 (NS)
Family history	_0.098	0.358 (NS)
History of previous Fracture	_0.329	0.002 (HS)
Smoking	0.028	0.793 (NS)

r: coefficient of Spearman correlation, NS: not significance at *p*> 0.05*;* HS: Highly significance at $P \leq 0.001$

3-4- Comparison of RANKL/OPG percentage in patients and control groups.

Detecting RANKL- OPG percentage in present research indicatedthat there was huge difference between two research groups. The median RANKL-OPG in osteoporosis rate was 105.64 (341.14) it wasconsiderably greater as per P<0.001 compared to that in control15.604 (21.07), according to table (9).

Table (9): Comparison of ratio of RANKL/OPG between healthy controls and patients with Osteoporosis.

	Case – control comparison		
RANKL/OPG	**Patients n = 50**	**Control n = 40**	***P***
Range	16.86– 1028.54	1.28 – 381.55	**< 0.001 † HS**
Median (IQR)	105.64 (341.14)	15.604 (21.07)	
Mean Rank	240.83	33.25	

n: casesnumber, IQR: interquartile range,†: Mann-Whitney U test, HS: Highly significance at $p \leq 0.001$

The OPG-RANKL-RANK pathway of signalinghas been established as a traditional pathway linked to osteoclastogenesis[39].

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Osteocytes are also stimulated by mechanosensory stimuli to generate a variety of proteins that influence bone resorption [40] RANKL also OPG are both mechanosensitive, but mice deficient RANKL from osteocyte are shielded from bone loss caused by inactivity[41].

Our results in agreement with other findingson ovarictomized ratsthat indicated noticeable expression in RANKL which lead to increasing in rate of RANKL to OPG while expression of OPG was not changed, on the other hand, production of OPG induced by estrogen and when OPG missing, the superior molecule is RANKL that share in formation of osteoclastsand then in lossing of bone [42].

Numerousearlier studies had discovered a variety of correlations;some have demonstrated OPG and RANKL to be independently associated with osteoporosis,whereas others have reported positive relationship of OPG versus negative relationship of RANKL toBMD [45].

Some of these studies have shown that OPG besides RANKL are both linkedto osteoporosis [43, 44] while some have discovered an OPG-positive, RANKL-negative correlation with BMD [45].

Our results also in agreement with other study that concluded that their findings reduced β-catenin in serum and a correspondingly larger RANKL-OPG proportion could be implicated in the pathogenesis of osteoporosis in postmenopausal women [46].

Generally,when RANKL transcription is elevated, OPG transcription is typically declined or not triggered to same extent as RANKL, ensuing in a shift in the RANKL/OPG equation in consideration of osteoclastogenesis [47].
The disease is primarily caused by cytokines such as TNF-α and IL-1, as well as cells including T and B cells. The osteoclastogenesis and eventual bone loss are also triggered by the RANK-RANK-OPG equation [48].

The RANKL-RANK-OPG regulatory structure is prone to engage in estrogen's antiresorptive behavior due to its crucial role in osteoclast synthesis and action.In vitro even in human researches, estrogen has appeared to maximize OPG expression of genes and synthesis of protein [49].

It would assist encourage osteoporosis medications unless the expression of gene mechanisms of the RANKL-OPG system could be properly defined at the gene transcriptional level.The role of DNA methylation in the OPG to RANKL cycle has currently been documented in human bone [50].

In the homeostasis of bone metabolism, the equilibrium between OPG and RANKL is essential.An imbalance in the OPG-RANKL level, on the other hand, can lead forlosing of skeletal mass[51].

The proportion of RANKL to OPG has been noticed in a variety of findings to influence remodeling of bone,while RANKL is considerable, bone turnover takes place; but at the other extreme, since OPG is greater, the construction of bone take priority.As a function of its

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capability to defend bone against intense resorption through combatting the osteoclastic impact of RANKL, OPG was named.Cytokines, hormones and growth factors that impair RANKL-OPG balance trigger metabolism disequilibrium of bone[52].

3-5- Correlation between IL-17, RANKL and OPG

Correlation of IL-17,OPG and RANKL are listed in (table-10). IL-17 level was showed highly significant positive correlation with RANKL as r=0.366, P= < 0.001, while significant negative correlation of OPG (r = 0.269, p = 0. 01).

Regarding association ofRANKL levels revealedconsiderable negative link to OPG levels(r=-0.350, p= 0. 01), table (7).

[Table 10]: Association ofIL-17, OPG, RANKL in patients with Osteoporosis and control subjects

Variables	IL-17	RANKL	OPG
IL-17	-	r = 0.366 p= < 0.001	r=-0.269 p= 0.01
RANKL	r = 0.366 p= < 0.001	-	r= - 0.350 p= 0.01
OPG	r = -0.269 p = 0.01	r = - 0.350 p =0.01	-

r: Spearman correlation coefficient, NS:for not-significance at *p*> 0.05, HS: Highly-significance at $p \leq 0.001$

3-6- Correlation between IL-17, RANKL and OPG

Findings of association among RANKL,OPG and IL-17 RANKL to OPG which as per (table-11). IL-17 level was indicated huge significance of positive correlation with RANKL (r is 0.366 but P is < 0.001) although significant negative correlation to OPG (r is -0.269 , p is 0.

Regarding the association of RANKL levels showed significant negative correlation withOPG levels(r=-0.350, p= 0. 01), (table -6). While the RANKL/OPG indicated highly significant positive correlation with IL-17 and RANKL (r=-0.416, p= < 0.001; r=-0.791, p= < 0.001) consequently, but valuable negative correlation with OPG (r=-0.777, p= < 0.001).

[Table 11]: Relation ofRANKL, OPG andIL-17 in patients with Osteoporosis and control subjects

Variables	IL-17	RANKL	OPG	RANKL/OPG
IL-17	-	r = 0.366 p= < 0.001	r = -0.269 p= 0.01	r = 0.416 p= < 0.001
RANKL	r = 0.366 p= < 0.001	-	r = - 0.350 p= 0.01	r = 0.791 p= < 0.001

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OPG	r = -0.269 p= 0.01	r = - 0.350 p= 0.01	-	r = - 0.777 p= < 0.001
RANKL/OPG	r = 0.416 p=< 0.001	r = 0.791 p= < 0.001	r = - 0.777 p= < 0.001	-

r: Spearman correlation coefficient, NS: not-significance-at $p > 0.05$, HS: highly-significance at $p \leq 0.001$

Several elements, namely TNF-α even cytokines of IL-17, IL-11, IL-6, and IL-1, have been recently settled to improve expressionof RANKL, comparably, the osteoclastogenic effects of IL-7, IL-6, and IL-1 have been stated to be mediated by RANKL expression [53].IL-17 is indeed an efficient osteoclastogenesis inducer [54] and renders osteoclast precursors further responsive to RANKL [55].

Our results agree with other studywhich had revealed markedly expandingin IL-17valuesaddition toserum inflammatory cytokines of women that 'postmenopaused' comparing to postmenopausal women but with osteopenia andwomen of premenopause[56]. Researchesproved thatIL-17 has direct action on osteoclasts,in spite of IL-17 can cooperate with other cytokines like RANKL, TNF-α and IL-1 in osteoclastogenesis besides IL-17 can induceclastogenesis of bones by stimulating expression of RANKL in osteoblasts and progress demineralization of bone[57].
Whilst also stimulating IL-1 plus TNF-α, IL-17 generate inflammation locally, expression ofRANKLand induction precursors of osteoclast cells[58].Chang *et al*. [59] discovered that proinflammatory IL-17 as well as TNF-α impede osteogenic MSCs differentiation.The relevance both RANKL with OPG to pathways on molecular levels in osteoporosis disease has been emphasized in survey, promoting concept of using them as therapy goals [60, 61,62].

Conclusion: According to our findings, RANKL, OPG and IL-17 in addition to RANKL to OPG proportion play an important role during pathogenesis of osteoporosis, as a result detection of these parameters alone and detection the association between them and between RANKL, OPG with IL-17,also the percentage of RANKL to OPGwhichassist in original diagnosis of disease like osteoporosis and can effectively provide an approach for osteoporosis detection.

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References:

[1]Yedavally-Yellayi, S., Ho, A. and Patalinghug, E.,(2019). Update on Osteoporosis. *Primary Care: Clinics in Office Practice*, 46(1), pp.175-190.
[2] Al-Hafidh A. H., Saeed G.T. and Goral, F. l.,(2019). Prevalence of Osteoporosis and Osteopenia in Iraqi Premenopausal and Postmenopausal Subjects among a Sample of Patients Attending Baghdad Teaching Hospital Article in Journal of Global Pharma Technology ISSN: 0975 -8542 Journal of Global Pharma Technology
[3] Einhorn TA., (1998). The cell and molecular biology of fracture healing. Clin OrthopRelat Res (355 Suppl): S7-21.

[4] Gerstenfeld LC, Cullinane DM, Barnes GL, et al. (2003), Fracture healing as apost-natal developmental process: Molecular, spatial, and temporal aspects ofits regulation. J Cell Biochem 88: 873-84.

[5] Looker AC, Bauer DC, Chesnut CH, et al. (2000).Clinical use of biochemical markersof bone remodeling: Current status and future directions. Osteoporos Int , 11: 467-80.

[6] Kurban S, Akpinar Z, Mehmetoglu I., (2008), Receptor activator of nuclear factorkappaB ligand (RANKL) and osteoprotegerin levels in multiple sclerosis. MultScler 14: 431-2.

[7] Ono T, Nakashima T.,(2018). Recent advances in osteoclast biology. Histochem Cell Biol;149:325–41.

[8] Trouvin A-P, Goeb V., (2010). Receptor activator of nuclear factor-kappa B ligand and osteoprotegerin: maintaining the balance to prevent bone loss. Clin Interv Aging;5:345–54.

[9] Zimmerman, S.M.; Heard-Lipsmeyer, M.E.; Dimori, M.; Thostenson, J.D.; Mannen, E.M.; O'Brien, C.A.; Morello, R., (2018). Loss of rankl in osteocytes dramatically increases cancellous bone mass in the osteogenesis imperfecta mouse (oim). Bone Rep., 9, 61–73.

[10] Panezai J, Ghaffar A, Altamash M, EngstroÈm P-E, Larsson A., (2018). Periodontal disease influences osteoclastogenic bone markers in subjects with and without rheumatoid arthritis. PLOS ONE 13(6): e0197235.

[11] Mori, G., et al. (2013). The Interplay between the bone and the immune system. *Clin Dev Immunol*, *p.* 720504.

[12] Pietschmann, P., et al.(2016). Immunology of Osteoporosis: A Mini-Review. Gerontology, 62(2): p. 128-37.

[13] Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, et al. (2006). Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. J Exp Med 203:2673–82.

[14] Ciucci, T., Ibáñez, L., Boucoiran, A., Birgy-Barelli, E., Pène, J., Abou-Ezzi, G., Arab, N., Rouleau, M., Hébuterne, X., Yssel, H., Blin-Wakkach, C. and Wakkach, A., (2014). Bone marrow Th17 TNFα cells induce osteoclast differentiation, and link bone destruction to IBD. *Gut*, 64(7), pp.1072-1081.

[15] Dar HY, Singh A, Shukla P, Rajaneesh A, Mondal RK, Mishra PK, et al. (2018), High dietary salt intake correlates with modulated Th17-Treg cell balance resulting in enhanced bone loss and impaired bone microarchitecture in male mice. Sci Rep, 8:2503.

[16] Komatsu N, Takayanagi H., (2012). Autoimmune arthritis: the interface between the immune system and joints. Adv Immunol 115:45–71.

[17] Raphael I, Nalawade S, Eagar TN, Forsthuber TG., (2015) . T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine 74:5–17.

[18] Adamopoulos IE, Chao CC, Geissler R, Laface D, Blumenschein W, Iwakura Y, et al. (2010) . Interleukin-17A upregulates receptor activator of NF-kappaB on osteoclast precursors. Arthritis Res Ther , 12:R29.

[19] Kim, B., Lee, K. and Park, B., (2018). Icariin abrogates osteoclast formation through the regulation of the RANKL-mediated TRAF6/NF-κB/ERK signaling pathway in Raw264.7 cells. *Phytomedicine*, 51, pp.181-190.

[20] Al-Masaoodi, R. A., Alaauldeen S. M., Al-Sallami, and Al-Baseesee, H., (2019). The relation between the RANKL and resistin in menopausal women with osteoporosis, AIP Conference Proceedings 2144, 040012.

[21] Mehansho, H., Majeti, S. and Tzeghai, G.E., Summit Innovation Labs LLC, (2019).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 3805 - 3821
Received 15 April 2021; Accepted 05 May 2021.

Treatment and Prevention of Joint Disorders. U.S. Patent Application 16/050,069.

[22]Ameen O., Yassien R. I. and Naguib Y.M.,(2020) . Activation of FoxO1/SIRT1/RANKL/OPG pathway may underlie the therapeutic effects of resveratrol on aging dependent male osteoporosis; BMC Musculoskeletal Disorders 21:375.

[23] de Villiers, T., (2015). The role of menopausal hormone therapy in the management of osteoporosis. *Climacteric*, 18(sup2), pp.19-21.

[24] Lorenzo J.A, Sousa S.L, Fonseca J.M, Hock, J.M, Medlock E.S., (2017).Colony stimulating factors regulate the development of multinucleated osteoclasts from recently replicated cells in vitro. *J Clin Invest*, 80:160-164.

[25] Metzger CE and Narayanan SA., (2019). The Role of Osteocytes in Inflammatory Bone Loss. Front. Endocrinol. 10:285.

[26] Abdallah BM, Stilgren LS, Nissen N, Kassem M, Jorgensen HR and Abrahamsen B., (2005).Increased RANKL/OPG mRNA ratio in iliac bone biopsies from women with hip fractures. Calcif Tissue Int. ;76:90–97.

[27] AwasthiH,Mani D, Singh D and Gupta A., (2018).The underlying pathophysiology and therapeutic approaches for osteoporosis. Med Res Rev.;1–34.

[28] Azizieh F. Y. , Shehab D., Al Jarallah K., Gupta R. and Raghupathy R.,(2019).Circulatory Levels of RANKL, OPG, and Oxidative Stress Markers in Postmenopausal Women With Normal or Low Bone Mineral Density, Biomarker Insights Volume 14: 1–7.

[29] Wang, P., Cao, Y., Zhan, D., Wang, D., Wang, B., Liu, Y., Li, G., He, W., Wang, H. and Xu, L., (2018). Influence of DNA methylation on the expression of OPG/RANKL in primary osteoporosis. *Int. J. Med. Sci.* Vol.15(13), pp.1480-1485.

[30] AL-Tai T.H., (2015). Serum IL-17 and postmenopausal osteoporosis, J Fac Med Baghdad; Vol.57, No .4

[31] Talaat, R. M., Sidek, A., Mosalem, A., and Kholief, A., (2015). Effect of bisphosphonates treatment on cytokine imbalance between TH17 and Treg in osteoporosis. Inflammopharmacology, 23(2–3), 119–125.

[32] Gillespie, M. T., Kho, A., Krastins, B., Sarracino, D. A., Thornhill, T. S., Chase, M., and Lee, D. M., (2007). Impact of cytokines and T lymphocytes upon osteoclast differentiation and function. Arthritis Research & Therapy, 9(2), 103.

[33] Ginaldi, L., De Martinis, M., Ciccarelli, F., Saitta, S., Imbesi, S., Mannucci, C., & Gangemi, S., (2015). Increased levels of interleukin 31 (IL-31) in osteoporosis. BMC Immunology, 16, 60.

[34] Molnar L, et al. (2014), High prevalence of increased interleukin-17A serum levels in postmenopausal estrogen deficiency. Menopause, 21(7): 749-752.

[35] Bluher S and Mantzoros G.S., (2009). Leptin in humans:lessons from translation research.*Am.J.Clin.Nutr*.;89:991-997.

[36] Weitzmann M.N and Pacifici R., (2005). The role of T lymphocytes in bone metabolism . *Immunol.Res*.;208:154-168.

[37] Jaya G, et al., (2009). Abone-protective role for IL-17 receptor signaling in ovariectomy-induced bone loss .*Eur.J.Immunol*.;39:2831-2839.P

[38]Marjolaine G and Gael Y.R., (2014).Inflammation and bone remodeling pathologies. International Journal of The paedics.;1(4).

[39] Wang, C., He, H., Wang, L., Jiang, Y. and Xu, Y., (2018). Reduced miR-144-3p expression in serum and bone mediates osteoporosis pathogenesis by targeting RANK. *Biochemistry and*

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 3805 - 3821
Received 15 April 2021; Accepted 05 May 2021.

Cell Biology, 96(5), pp.627-635.

[40] You L, Temiyasathit S, Lee P, Kim CH, Tummala P, Yao W, et al., (2008).Osteocytes as mechanosensors in the inhibition of bone resorption due to mechanical loading. Bone. 42:172–9.

[41]Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA.,(2011). Matrix-embedded cells control osteoclast formation. *Nature Med.* 17:1235–42.

[42] Khera, A., Kanta, P., Kalra, J., Dumir, D. and M, T., (2018). Resveratrol restores the level of key inflammatory cytokines andRANKL /OPG ratio in the femur of rat osteoporosis model, *Journal of Women & Aging*, 31(6), pp.540-552.

[43] Mezquita-Raya, P., de la Higuera, M., García, D., Alonso, G., Ruiz-Requena, M., de Dios Luna, J., Escobar-Jiménez, F. and Muñoz-Torres, M., (2005).The contribution of serum osteoprotegerin to bone mass and vertebral fractures in postmenopausal women. *Osteoporosis International*, 16(11), pp.1368-1374.

[44] Jabbar S, Drury J, Fordham JN, Datta HK, Francis RM, Tuck SP., (2011).Osteoprotegerin,RANKL and bone turnover in postmenopausal osteoporosis. *J Clin Pathol*.;64:354–357.

[45] Stern A, Laughlin GA, Bergstrom J, Barrett-Connor E., (2007). The sex-specific association of serum osteoprotegerin and receptor activator of nuclear factor κB legend with bone mineral density in older adults: the Rancho Bernardo study. *Eur J Endocrinol*.;156:555–562.

[46] Xu, X., Shen, L., Yang, Y., Zhu, R., Shuai, B., Li, C. and Wu, M., (2013). Serumβ-Catenin Levels Associated with the Ratio of RANKL/OPG in Patients with Postmenopausal Osteoporosis. Hindawi Publishing Corporation, *International Journal of Endocrinology*, Article ID 534352, pp.1-7.

[47] Boyce B.F. and Xing L., (2008). Functions of RANKL/ RANK/OPG in bone modeling and Remodeling , Arch Biochem Biophys. 473(2): 139-146.

[48] Xie Y., Hu C., Feng Y., Li D., Tingting Ai, Huang Y., Chen X., Huang L. and Tan J.,(2020).Osteoimmunomodulatory effects of biomaterial modification strategies on macrophage polarization and bone regeneration, *Regenerative Biomaterials*, 233–245.

[49] Eghbali-Fatourechi G., Lacey D.L. and Riggs B.L., (2003). Role of RANK ligand in mediating increased bone resorption in early postmenopausal women *J Clin Invest.*111(8):1221-1230.

[50] Delgado-Calle J, Sanudo C, Fernandez A F, et al., (2012).Role of DNA methylation in the regulation of the RANKL-OPG system in human bone. *Epigenetics-Us.*7: 83-91.

[51] Han X., Gong S., Li N., Wang X., Liu P., Xu Y., He X., Jiang W. and Si S.,(2019). A Novel Small Molecule Which Increases Osteoprotegerin Expression and Protects Against Ovariectomy-Related Bone Loss in Rats. *Front. Pharmacol*. 10:103.

[52] Kapasa E.R., Giannoudis P.V., Jia X., Hatton P.V. and Yang X.B., (2017).The Effect of RANKL/OPG Balance on Reducing Implant Complications, *J. Funct. Biomater.*8, 42.

[53] AwasthiH.,ManiD., Singh D., Gupta A., (2018). The underlying pathophysiology and therapeutic approaches for osteoporosis. *Med Res Rev*.1–34.

[54] Kotake S., Udagawa N., Takahashi N., Matsuzaki K., Itoh K., Ishiyama S., et al.,(1999). IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J Clin Invest.* 103:1345–52.

[55] Adamopoulos IE., Chao C., Geissler R., Laface D., BlumenscheinW., Iwakura Y., et al., (2010). Interleukin-17A upregulates receptor activator of NF-kB on osteoclast precursors.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 3805 - 3821
Received 15 April 2021; Accepted 05 May 2021.

Arthr Res Ther. 12:R29.

[56] Zhang, J., Fu, Q., Ren, Z., Wang, Y., Wang, C., Shen, T., Wu, L., (2015). Changes of serum cytokines-related Th1/Th2/Th17concentration in patients with postmenopausalosteoporosis. Gynecological Endocrinology: The Official Journal of the International Society of Gynecological Endocrinology, 31(3), 183–190.

[57] Gaffen, S. L., Jain, R., Garg, A. V.and Cua, D. J., (2014). The IL-23-IL-17 immune axis: From mechanisms to therapeutic testing. Nature Reviews. Immunology, 14(9), 585–600.

[58] Lubberts E., (2008). IL-17/Th17 targeting: on the road to prevent chronic destructive arthritis? Cytokine ;41:84–91.

[59] Chang J., Liu F., Lee M. et al., (2013). NF-kappa B inhibits osteogenic differentiation of mesenchymal stem cells by promoting beta-catenin degradation.*Proc Natl Acad Sci*. U S A;110:9469–9474.

[60] Nabipour I., Larijani B., Vahdat K., et al., (2009). Relationships among serum receptor ofnuclear factor-κB ligand, osteoprotegerin, high-sensitivity C reactive protein and bone mineral density in postmenopausal women: osteoimmunity versusosteoinflammatory. Menopause;16:950–955.

[61]Rogers A. and Eastell R., (2005).Circulating osteoprotegerin and receptor activator for nuclear factor kappaB ligand: clinical utility in metabolic bone disease assessment. *J Clin Endocrinol Metab*.;90:6323–6331.

[62] Walsh MC. and Choi Y., (2014).Biology of the RANKL–RANK–OPG system in immunity, bone, and beyond. *Front Immunol*.;5:511.