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Evaluating the clinical significance of Osteoprotegerin Serum Levels as a Predictive Marker in Rheumatoid Arthritis

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ABSTRACT

Laboratory tests such as the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and Immunoglobulin M (IgM) have been used as markers of inflammation and disease activity in rheumatoid arthritis (RA), although there is still no clear consensus on when to use one, the other, or all. We aimed to evaluate the level of OPG in RA patients compared with control in order to estimate the predictive value of OPG. Eighty five patients with active RA, attending rheumatology department at Al-Sadder General Hospital in the province of Maysan-Iraq, diagnosed according American College of Rheumatology (ACR) revised criteria were included. The patients' tender and swollen joint counts were calculated. Laboratory investigations were done including ESR by Westergren method, CRP, IgM and OPG by ELISA method, assessment of disease activity using DAS28 score. A non-significant change ($p>0.05$) was seen in the level of BMI, significantly increased ($p<0.01$) in levels of ESR, CRP, IgM, and OPG as compared to the control group. All patients showed disease activity at the time of the study, their DAS28 scores ranged from 5.20 to7.40 (Mean ±SD 6.43±0.80). There was a positive and highly significant ($p< 0.01$) correlation between DAS28 values and ESR, CRP, IgM, and OPG values (r-value was 0.662, 0.695, 0.689, and 0.636, respectively). The OPG was more specific than Rf (IgM, CRP, and ESR) in the diagnosis of RA. So it could be a useful assay in establishing the diagnosis of RA, especially in ambiguous cases or RF negative patients with RA, and also a better predictor of disease severity.

1. Introduction

Rheumatoid arthritis (RA) is a chronic disease marked by systemic inflammation and progressive periarticular bone loss, which leads to joint-related impairment. In the synovia of RA patients, activated macrophages, T- and B-lymphocytes, dendritic cells, and other immunocompetent cells can be seen [1]. These cells also play a role in bone and cartilage degeneration by producing proinflammatory cytokines

such as tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon (IFN-γ) [2]. These cytokines also target and impact bone metabolism in RA, in addition to enhancing inflammatory cascades. This is accomplished by influencing the expression of important regulatory proteins like osteoprotegerin (OPG) [3].

OPG is a tumor necrosis factor receptor that acts as a soluble decoy receptor for the receptor activator of nuclear factor-ligand (RANKL) to prevent osteoclast activation and bone resorption. OPG has 401 amino acids in its molecule; however, breaking a single 21-amino-acid peptide results in the formation of a mature 380-amino-acid form with four amino-terminal cysteine-rich domains that are structurally similar to the extracellular parts of other TNF receptor partners. Sections 5 and 6, which are death domain symmetrical portions, are mixed together at the carboxyterminal [4]. The biological effect of the OPG molecule is controlled by three structural scopes. The N-terminal part contains a cysteine-rich zone that is important for dimerization and osteoclastgenesis, while the C-terminal section has a death domain and a heparin-binding domain. It's upregulated in calcified coronary plaques and linked to the severity of angiographic disease and cardiovascular events in the absence of traditional risk factors [5]. In the inflamed synovium of individuals with rheumatoid arthritis, however, OPG is produced by osteoblasts, dendritic cells, and -cells, according to some recent research (RA). The ability of RA macrophages to develop into osteoclasts is dependent on RANKL, according to these findings. Furthermore, RANKL was found to be expressed by activated RA synovial fibroblasts [6]. The inflammatory infiltrate in the RA synovium also contains OPG-producing dendritic cells and B-cells, which inhibit osteoclast production and activation. Furthermore, OPG levels in RA joint effusions are lower than in other joint disorders. As a result, it appears to play a key role in preventing RA-related erosions and osteoporosis [7].

The high specificity of the RA checking still needs more deeply studies, so this study comes to assess level of OPG in RA patients compared with control in order to estimate the predictive value of OPG.

2. MATERIALS AND METHODS

2.1 Study population and selection of patients

Between December 2020 and the end of May 2021, samples were obtained from "the outpatient rheumatology unit" at Al-Sadder General Hospital in the province of Meisan-Iraq. In this case-control study, 104 obese volunteers between the ages of 40 and 71 years old with definite RA according to the ARA (American Rheumatism Association) criteria, [8] of whom were obese, took part. The participants were split into two groups, as follows: Forty-one patients (13 men and 28 women) with arthritis were paired with forty-four healthy blood donors (20 men and 24 women) who had no history of arthritis. While 19 volunteers (13 sick and 6 healthy controls) were omitted from the study due to the inability to conduct a follow-up investigation. All patients were asked about their personal characteristics, clinical characteristics (RA onset, early symptoms, categorization criteria, extra-articular illness, and radiographic signs), and drugs taken. Patients were also quizzed on their capacity to carry out daily tasks.

2.2 Collection of blood samples

After a 12-hour fast and 30-minute rest in the supine posture, all blood samples were taken in the morning between 09:00 and 09:30 hours. Five ml venous blood was collected from each subject, 1 ml was delivered into ESR tube and the rest of the sample was delivered into a tube without anticoagulant. The ESR tube was left for 60 minutes at room temperature to measure ESR (mm/h). The second tube was placed in a centrifuge and spun at 402 x g for 10 minutes and then the serum separated and were stored in deep freezing at (-20ºC) until using.

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2.3 Clinical assessment

Patients were given a detailed medical history, a thorough clinical examination, and locomotor examinations. A rheumatologist evaluated the tender joint count (TJC) and swollen joint count (SJC) of RA patients. The disease activity score in 28 joints was used to determine disease activity at the time of our investigation (DAS28) [9]. The healthy control and patient's body mass index was calculated as the following formula [BMI (kg/m^2) = Wt in kg / Ht in m^2] [10]. Level of serum OPG was measured using a sandwich-type-ELISA based on OPG specific antibodies (BT-Lab, Shanghai, China, Cat.No.: E1558Hu) kit, IgM RF was determined using the (Abnova- KA4880/Taiwan) kit and CRP was determined using the (Abnova- KA1052/Taiwan) kit.

2.4 Statistical analysis

SPSS software version 21 was used for statistical analysis (IBM Corporation, New York, USA). The statistical significance was determined using the student t-test. Pearson correlation was used to determine the correlations between the variables. Significant differences were defined as $p<0.05$ and $p<0.01$, respectively.

3. Results

The general demographic characteristics of all volunteers participated in this work were presented in Table 1.

Table-1: The demographic characteristics of the present study.

The characteristics		Healthy Control	Rheumatoid arthritis Patients
Total (No.)		44	41
Age (mean ± SD)		47.64± 3.87	48.86 ± 5.32
Gender, (%)	Male	20 (45.45%)	13 (31.70%)
	Female	24 (54.54%)	28 (68.29%)
Demographic area, (%)	Urban	34 (77.27%)	38 (92.68%)
	Rural	10 (22.72%)	3 (7.31%)
Educational Background, (%)	Learned	33 (75.00%)	36 (87.80%)
	Illiterate	11 (25.00%)	5 (12.19%)
Employment Status, (%)	Employed	29 (65.90%)	31(75.60%)
	Not Employed	15 (34.09%)	10 (24.39%)
Food Habits, (%)	Vegetarian	12 (27.27%)	7 (17.07%)
	Non-Vegetarian	32 (72.72%)	34 (82.92%)
Disease duration, (%)	< 5 year	-	30 (73.17%)
	> 5 year	-	11 (26.82%)
Smoking habit, (%)	Negative	-	37 (90.24%)
	Positive	-	4 (9.75%)
Family history, (%)	-		8 (19.51%)

Extra-articular manifestation, (%)	-	14 (34.14%)
Radiological manifestation (erosive), (%)	-	18 (43.90%)
Drug treatment (DMARD or steroid or anti TNF drugs), (%)	-	36 (87.80%)

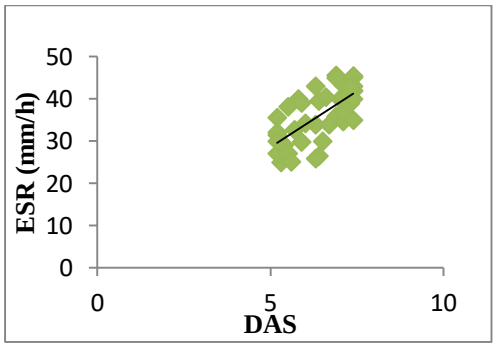
Compared with normal control, the results indicated that rheumatoid patients had a non-significant change ($p>0.05$) was seen in the level of BMI and increased levels of ESR, CRP, IgM and OPG ($p< 0.01$), as shown in Table 2. Furthermore, same table 2 reflect that all patients showed disease activity at time of the study, their DAS28 score was ranged from 5.20 to7.40 (Mean ±SD = 6.43±0.80).

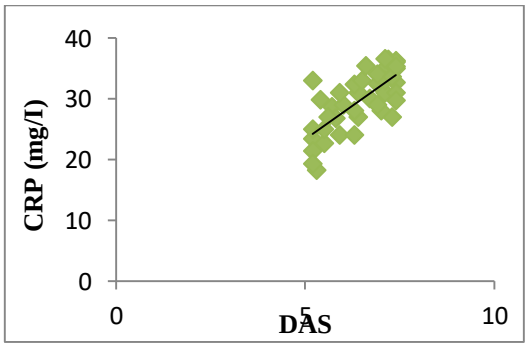
Table-2: The levels of total parameters measured in the present study.

Parameters	Rheumatoid arthritis patients n=41						Healthy Control n=44	p-Value
	Mean ±SD	SE	Median	Range	95 % C.I			
					Lower	Upper	Mean ± SD	
BMI (Kg/m^2)	30.13± 0.50	0.078	29.99	29.76-32.23	29.97	30.29	29.98±0.32	p>0.05
ESR (mm/h)	36.10± 6.23	0.97	37.00	24.78-45.43	34.13	38.06	8.94 ±1.34	p< 0.01
CRP (mg/I)	29.68±4.84	0.75	30.00	18.23– 36.54	28.15	31.21	5.67±1.63	p< 0.01
IgM (U/ml)	92.90 ± 7.97	1.24	94.00	77.40–102.67	90.39	95.42	9.12 ± 2.16	p< 0.01
OPG (ng/mL)	4.19± 0.57	0.089	4.32	2.98 – 5.10	4.01	4.37	2.51± 0.23	p< 0.01
Tender joint count	15.00 ± 0.44	0.068	15.10	14.03 – 16.12	14.95	15.23	NA	
Swollen joint count	12.00 ± 1.07	0.16	12.00	10.10 – 15.20	1.73	12.40	NA	
DAS28	6.43± 0.80	0.12	6.50	5.20 – 7.40	6.18	6.68	NA	

Data are presented as mean ± SD, SE: Standard Errors; n: Number of the subjects; Range: is the difference between the highest and lowest values in the set; 95% C.I: Confidence limits (Lower and Upper); NA: not analyzed.

DAS28 values were correlated with variable clinical and laboratory data in the studied group. The present results confirmed that there were a positively and highly significantly ($p< 0.01$) correlation between DAS28 values and ESR, CRP, IgM and OPG values (r value was 0.662, 0.695, 0.689 and 0.636, respectively), as shown in Figure 1.





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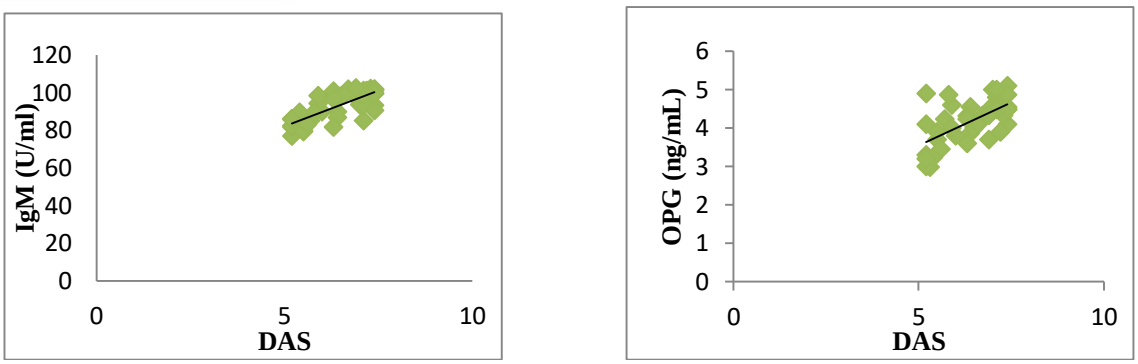

Figure-1: Correlation coefficient (r) of DAS (DAS) with ESR, CRP, IgM and OPG in rheumatoid arthritis patients.

4. DISCUSSION

In order to provide adequate treatment to people with RA, it is critical to accurately quantify disease activity. Because it integrates changes in joint counts, global responses, and the ESR, the Disease Activity Score (DAS 28) is the most often used metric for disease activity evaluation (or CRP). As a result, it can be used in both clinical trials and everyday practice to assess the absolute level of disease activity and response to treatment [11]. The more active the arthritis is, the higher the DAS 28 score >5.1. Our patients exhibited a mean (6.3), indicating a high level of active illness.

Disease activity score (DAS) and its variations include ESR or CRP as part of their score, and as a result, their use and discussion in disease activity evaluation has expanded. ESR and CRP may play a disproportionately large impact in the overall score due to the way these indices are calculated [12].

ESR, CRP, and IgM all tend to correlate with RA disease activity as well as disease severity, therefore they could be valuable for evaluating therapy response [5,6]. Our patients had higher levels of C-reactive protein (CRP) (29.684.84 vs 5.671.63 mg/I), erythrocyte sedimentation rate (ESR) (36.10 6.23 vs 8.941.34 mm/h), and immunoglobulin M (IgM) (92.90 7.97 vs 9.12 2.16 U/ml) than healthy controls, indicating disease activity and radiologic progression in rheumatoid arthritis. Because ESR is a mirror of fibrinogen levels in the blood, diseases that elevate fibrinogen can boost ESR, even if they aren't considered inflammatory. Pregnancy, diabetes, end-stage renal disease, and heart disease are among them. Increased sedimentation is also caused by large increases in the concentration of a single molecular species, such as a monoclonal immunoglobulin in multiple myeloma10. Microcytosis, polycythemia, and irregularly shaped RBCs (such as sickle cells and spherocytes) obstruct aggregation and reduce the ESR [13].

Furthermore, the increased ESR, CRP, and elevated IgM and DAS28 levels seen in RA patients could indicate that it plays a role in disease pathogenesis in these patients. By reacting with IgG, autoantibodies like IgM-RF are particularly effective complement activators. This activation causes synovial cells to produce inflammatory cytokines, which can cause inflammation, cartilage degradation, and bone erosion [14].

TNF receptor OPG is a soluble member of the TNF receptor family. It's made by osteoblastic cells and serves as a RANKL receptor antagonist, preventing osteoclast precursor cells from differentiating and mature osteoclasts from activating. High levels of OPG have been shown to be osteoprotective in recent mouse experiments [15]. OPG-knockout animals, on the other hand, had increased osteoclast activity and developed severe osteoporosis. Activated CD4-positive T-lymphocytes create RANKL in RA, which causes osteoclast activation and hence bone lesions. OPG has the ability to entirely stop this procedure [16].

Furthermore, because of the upregulation of numerous cytokines in RA, not only RANKL, but also OPG, to a lesser extent, may be raised. Other cells, such as endothelial cells and fibroblasts, can, nevertheless, produce OPG. As a result of the activation of various additional cells, patients with active inflammation may have higher OPG readings [17]. Furthermore, the amount of OPG in people with RA is much higher than in people who are healthy. In addition, patients with active RA, as characterized by a high ESR and a high rheumatoid factor, had significantly higher OPG levels as determined by analysis of variance. Serum OPG levels were also found to be considerably higher in females and to increase with age in a large population of healthy controls [18]. As a result, the higher levels identified in older healthy people were significantly lower than the mean value found in our study. On the other hand, we found no evidence of age dependence in our small cohort of RA patients who had already experienced considerable increases in disease activity levels [19].

Our findings could be regarded as a mechanism for upregulation of OPG synthesis in patients with active RA in response to an increase in RANKL and/or sRANKL. According to several research findings, RANKL expression was shown to be elevated in tissues surrounding bone erosions, but OPG was noticeably lacking in tissues of individuals with active RA [20].

In the current study, there was a substantial association between CRP and disease activity, which is consistent with a prior study, indicating that serum CRP is the most relevant biochemical marker for evaluating the disease activity of RA patients. Some studies, on the other hand, found that ESR, CRP, and IgM levels were only moderately linked with disease activity markers. These findings showed that a closer examination of the role of ESR, CRP, and IgM as inflammatory markers in RA patients seen in normal care should be warranted [21]. A substantial association between OPG and disease activity, on the other hand, could be an indirect signal of active processes in affected joints, regardless of whether the elevation is due to complexed OPG (in which case complexed blood-RANKL would be raised as well) or free OPG. Locally greater levels of RANKL cause osteoclast activation and bone degradation, which is apparently not entirely balanced by local OPG. Therefore, our findings may be consistent with those of [22], who found that the inflammatory infiltrate in the RA synovium contains not only RANKL-expressing T-cells, but also OPG-producing dendritic cells and B-cells. Furthermore, a rise in OPG serum levels could be the result of a combination of enhanced synovial OPG synthesis and an extra systemic reaction to counteract circulating sRANKL [23].

5. Conclusion

In conclusion, we believe that ESR, CRP, and IgM are not feasible markers in the clinical environment for monitoring inflammatory activity in RA patients, and that their use and reliance as measures of inflammation in RA patients should be reconsidered. In the diagnosis of RA, OPG was more specific than Rf (IgM, CRP, and ESR). Therefore, it could be a valuable assay for determining the diagnosis of RA, particularly in equivocal instances or people with RA who are RF negative, as well as a better predictor of disease severity. As a result, more research on a bigger scale with the same population is needed to verify that OPG levels may be used to predict remission or clinical response. A combination of these assays, on the other hand, may serve as a "gold standard" in the diagnosis of RA patients, as well as a superior predictor of disease severity.

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