ORIGINAL ARTICLE

Osteoprotegerin (OPG) and Matrix Gla protein (MGP) in rheumatoid arthritis patients: Relation to disease activity

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KEYWORDS
Rheumatoid arthritis (RA); Matrix Gla protein (MGP); Osteoprotegerin (OPG); Disease activity score 28-CRP (DAS28-CRP)

Abstract  Background: Imbalanced Matrix Gla protein (MGP) and Osteoprotegerin (OPG) levels occur in inflammatory diseases.

Aim of the work: The aim of the present study was to evaluate serum MGP and OPG levels in Rheumatoid Arthritis (RA) patients and study their relation to the disease activity.

Patients and methods: Forty-five female RA patients and 45 age and sex-matched healthy controls were included in this study. Disease activity score 28-C-reactive protein (DAS28-CRP) was used for the assessment of disease activity. High-sensitivity C-reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), MGP and OPG were measured in patients and controls. The associations of MGP and OPG with DAS28-CRP and the other laboratory and clinical variables were analyzed.

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1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease with polychartilaginous synovitis. It is characterized with the breakdown of cartilage, juxta-articular bone, and generalized bone loss with reduced bone mass. The consequences of this intense bone loss are painful joint deformities, progressive functional disability, and increased risk of bone fractures and increased mortality rates [1].

Receptor activator of nuclear factor κB ligand (RANKL) that belongs to the TNF superfamily, exists as a soluble form (sRANKL) [2,3]. Receptor activator of nuclear factor κB ligand, expressed on osteoclasts/stromal lineage cells, plays a stimulating role to transduce differentiation and send activation signals to osteoclast lineage cells through binding to its receptor (RANK) which leads to osteoclastogenesis and bone resorption in patients with RA [1,3]. Osteoprotegerin (OPG) is produced by osteoblasts and it is an important regulator of osteoclast development and function. Osteoprotegerin acts as a decoy receptor by blocking RANKL–RANK binding preventing osteoclastogenesis and bone resorption in mice, while OPG deletion leads to an increase in remodeling of bone and osteoporosis [6,7]. Thus, expression of RANKL and OPG give rise to controlling RANK activation and balance between OPG and RANKL levels; meanwhile, OPG plays fundamental roles in order to determine the extent of bone resorption [8–10]. Previous studies reveal that disequilibrium of RANKL and OPG together may indicate relatively skeletal complications as well as deregulations of this system implicate the pathophysiology of bone remodeling in patients with RA [11–13].

Several experiments clearly show the role of RANKL in inflammatory joint disease in laboratory animal models. Activated T cells in vivo culminate in RANKL-mediated increase in osteoclastogenesis and bone loss [14–16]. In RA, it has been confirmed that T-cell activation could cause osteoclastogenesis within the synovium and this can be conducted via two mechanisms: (A) Secretion of RANKL by active T cells in inflamed joints. (B) Increasing bone erosions. On the contrary, OPG probably contributes to preventing inflammation-induced bone resorption in RA patients [17]. Administration of OPG to animals with arthritis blocks bone demolition; however, with less influence on inflammation [18]. Serum OPG concentrations are also elevated in RA patients [19]. OPG is an effective inhibitor for differentiation of osteoclasts and could prevent bone resorption in patients with RA [20]. Some evidences suggest that the balance between OPG and RANKL is important in prevention of joint destruction [21,22] and therefore, data suggest that inhibition of RANKL function via OPG plays a protective role to bone destruction in RA patients [23].

Vascular calcification (VC) is known to be associated with a high risk of coronary atherosclerosis morbidity and mortality in RA patients [24]. Matrix GlA protein (MGP) is among the most important inhibitors of VC [11,25]. Its effect on VC is mediated by inhibiting of calcium crystal formation, with binding surplus calcium ions or small crystals in tissues and clearing them from circulation [26,27]. Data demonstrate that serum MGP concentrations in patients with calcification were lower in comparison to patients without calcification [28,29]. Herrmann et al. [30] reported a significant decrease in serum MGP level with increased severity of coronary calcification. Moe et al. [31] believe that OPG and MGP may protect against VC in the uremic patients. Nevertheless studies including correlations between serum OPG and MGP levels and disease activity score including C-reactive protein (DAS28-CRP) in patients with RA have not yet been reported.

The aim of the present study was to evaluate serum MGP and OPG levels in female Rheumatoid Arthritis (RA) patients and study their relation to the disease activity.

2. Patients and methods

Forty-five women aged 46.5 ± 12.6 years affected by RA (Disease duration: 1–22 years) were compared with 45 healthy age, sex and body mass index (BMI) matched controls (43.2 ± 9.2 years). The median and range of the age was 48 (21–69) years in the RA group and 41(21–62) years in the healthy control group (p = 0.2). Patients were selected consecutively from the Rheumatology Clinic of Tabriz University of Medical Sciences (RCTUMS) between August 2010 and Jan 2011 and were enrolled into the study according to the ACR/EULAR 2010 classification criteria of RA [32]. Before investigation, written informed consent was obtained from all participants. Exclusion criteria in the present study were patients with history of smoking or alcoholism, renal disease, cardiovascular and liver inheritance systemic disease, uncontrolled hypertension, nephrotic syndrome, diabetes mellitus, Cushing syndrome, thyroid disorders, anti-conception drugs and metabolic diseases. The patients with changed drug treatment schedule in the previous two months and during the
study period were also excluded. Conventional therapies of the patients were Prednisone (5–10 mg/day), Methotrexate (10–25 mg/week), Sulfasalazine (1–3 g/day), Hydroxychloroquine (200–400 mg/day), Cyclosporine (100–300 g/day), Azathioprine (100–150 mg/day), non-steroidal anti-inflammatory drugs (short-term interventions for control of Mechanical pains): Naproxen (250–500 mg/day), Ibuprofen (400–800 mg/day) and Diclofenac (25–75 mg/day). The ethics committee of Tabriz University of Medical Sciences reviewed and approved the study which is in compliance with the Declaration of Helsinki.

2.1. Clinical assessments

The clinical examination was done by a rheumatologist and DAS28-CRP was calculated according to the formula that is composed of the number of tender and swollen joints, patient’s global assessment of disease activity on a visual analogue scale (VAS) and CRP. Erythrocyte sedimentation rate (ESR) tends to reflect disease activity of the past few weeks, whereas CRP reflects more short-term changes in disease activity. Therefore, the advantage of CRP is that it is more sensitive to reflect disease activity of the past few weeks, whereas ESR can be influenced by confounding factors such as age, sex, fibrinogen levels, hypergammaglobulinemia, rheumatoid factor and anemia. For these reasons, DAS28-CRP using CRP instead of ESR was used in the present study [33].

2.2. Sample collection and analysis

All participants underwent blood sampling after 8 h fasting. All samplings were performed in a period of 4 weeks. The separated sera were collected and stored in −70 °C until laboratory tests were done. The serum concentration of MGP was measured by the Enzyme-Linked Immunosorbent Assay (ELISA) employing commercial kits. (Usen Life Science Inc, Wuhan, China, Lot. No.:101018140, Detection range: 39–2500 pg/ml, the sensitivity of the assay, or lower limit of detection was defined as the lowest protein concentration that could be differentiated from zero). The assay has high specificity for detection of human MGP and no significant cross-reactivity or interference between human MGP and analogues was observed. The serum concentration of OPG was measured by ELISA employing commercial kits [Boster Immunoleader, Wuhan, China, and Lot. No.:199656, Detection range: 93.8–6000 pg/ml, sensitivity: > 5 pg/ml. The assay has high specificity for detection of human OPG and no detectible cross-reactivity with any other cytokine was observed]. Anti-CCP was measured by the Medizym anti-CCP Ref ELISA kit (MEDIPAN GMBH, Ludwig-Erhard-Ring 3, 15827 Dahlewitz/Berlin–Germany, the analytical sensitivity was established to be 1.2 U/ml and the functional sensitivity was measured as 20% of inter-assay CV at about 2 U/ml). The concentration of hs-CRP was determined by immunoturbidometry assay (Pars Azmoon Co, Tehran, Iran). Serum creatinine (Cr), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total cholesterol (TC), triglyceride (TG), total calcium and phosphorus were measured by the enzymatic colorimetric method (Pars Azmoon Co, Tehran, Iran) with an automated chemical analyzer (Abbott analyzer, Abbott laboratories, Abbott Park, North Chicago, IL). The ESR was measured using whole blood and complete blood counts with differential counts analyzed by the H1-Technicon blood cell counter. All case and control samples were analyzed on the same day for each assay kit assay kit.

Statistical analysis: Statistical analyses were performed by SPSS software version 18.0 (SPSS Ins, Chicago, IL). The Kolmogorov–Smirnov test was used to evaluate data distribution. Results are expressed as median (minimum–maximum values), or mean ± SD and Mann–Whitney U test or independent t-test was used, as appropriate, to assess the significance of any differences between the two groups. All correlations were evaluated using the Spearman test and the statistical significance was set at P < 0.05.

3. Results

Ninety subjects; composed of 45 patients with RA and 45 healthy controls, participated in this study. Disease duration, components of disease activity, anti-cyclic citrullinated peptide (anti-CCP), complete blood count (CBC) and medications received by the rheumatoid arthritis patients are shown in Table 1. Other demographic and laboratory features of the patients and control are presented in Table 2.

Osteoprotegerin (OPG) did not correlated significantly with MGP in RA patients (r = 0.03, p = 0.86). Also, no significant correlations were found between the MGP and OPG with the hs-CRP level in these patients (r = 0.24, p = 0.12; r = 0.18, p = 0.23; respectively). Age positively correlated with OPG

| Table 1 Disease duration, factors of disease activity, anti-cyclic citrullinated peptide (anti-CCP), complete blood count (CBC) and medications received by the rheumatoid arthritis patients. |
|---|---|
| Variables | Rheumatoid arthritis patients (N = 45) |
| Disease duration (years)a | 4.5 (1–22) |
| Disease activity: | |
| Swollen joint counta | 2 (0–10) |
| Tender joint counta | 2.8 (0–12) |
| ESR (mm/60 min)b | 24.1 ± 14.3 |
| DAS28-CRPb | 2.5 ± 0.7 |
| Anti-CCP (unit)a | 149.5 (0.1–2290) |
| CBC: | |
| WBC (mm³/l)a | 7913.8 (3400–12,000) |
| Hemoglobin (g/dl)b | 12.5 ± 1 |
| Platelet (mm³/l) | 250346 ± 64,524 |
| Medications: [n (%)] | |
| Prednisone | 42 (93.3) |
| Methotrexate | 36 (80) |
| Sulfasalazine | 8 (17.6) |
| Hydroxychloroquine | 21 (46.7) |
| Cyclosporine | 1 (2.20) |
| Azathioprine | 3 (6.7) |
| NSAIDs | 2 (4.4) |

a Data are expressed as median (minimum–maximum).
b Values were expressed as the mean ± standard deviation (SD).

Rheumatoid Arthritis is among the most common inflammatory joint disorders, which is associated with systemic inflammatory mediators [34]. Bone destruction is a main unsolved problem in patients with RA. Published data have shown the prevalent role of the OPG/RANK/RANKL triad in bone pathophysiology. RANKL is the main osteoclast-stimulating factor and many clinical studies indicated that increased RANKL and/or decreased OPG, a soluble neutralizing receptor for RANKL, could develop bone erosions. So, bone resorption could be significantly reduced by inhibition of RANK as the receptor of RANKL and also by increasing OPG level [35–37].

The results of the present study showed that RA patients had significantly higher serum OPG and hs-CRP concentrations than healthy controls. Conflicting results have been suggested about prevention or induction role of OPG for arterial calcification [35–37]. Although OPG de novo could prevent arterial calcification, its secretion secondarily to inflammatory processes could mediate arterial calcification [38]. The mechanism seems secondary to the expression and up regulation of endothelial OPG, which is among the TNF alpha super family [39]. Evidences also suggest the role of OPG as a proinflammatory molecule and inducer of vascular calcification and atherosclerosis in RA [39–41].

In our study no significant correlations between OPG, MGP, and hs-CRP were found in the RA patients, which does not prove the results reported by Rhee et al. [42] who showed an association of OPG with CRP and acute phase reactants. The reason for this discrepancy may be due to different population groups; the high CRP level in their study was in the patients with coronary artery disease. These patients had a consistent situation without waxing and waning of inflammation, in contrast to our RA patients who did not have a constant inflammation milieu. Survival of OPG would be prolonged after binding to the Immunoglobulin-FC fragment [43]. It may also occur in patients with RA. The fluctuation of CRP is not a reliable biomarker on one hand, and non-decayed OPG on the other hand may be responsible for the non-significant correlation between hs-CRP and OPG [39].

Several investigators reported an excess of cardiovascular morbidity and mortality in active RA and the majority of car-

### Table 2  Demographic and laboratory characteristics of rheumatoid arthritis patients and controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>RA patients (n = 45)</th>
<th>Controls (n = 45)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic features:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.5 ± 12.6</td>
<td>43.2 ± 9.2</td>
<td>0.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.7 ± 3.2</td>
<td>22.0 ± 2.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>86 ± 9</td>
<td>74 ± 10</td>
<td>0.02</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>146 ± 34</td>
<td>112 ± 9</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Laboratory investigations:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (Iu/l)</td>
<td>20.8 ± 10.3</td>
<td>10.6 ± 2.8</td>
<td>0.01</td>
</tr>
<tr>
<td>AST (Iu/l)</td>
<td>22.8 ± 9.3</td>
<td>11.0 ± 3.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>217.6 ± 38.9</td>
<td>172.6 ± 13.9</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>142.7 ± 26.5</td>
<td>136.0 ± 32.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>4.2 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.5 ± 0.6</td>
<td>9.0 ± 0.5</td>
<td>0.002</td>
</tr>
<tr>
<td>OPG (pg/ml)</td>
<td>260.2 (18.8–3170)</td>
<td>69.4 (5.8–384)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(408.3 ± 520.9)</td>
<td>(92.5 ± 86.3)</td>
<td></td>
</tr>
<tr>
<td>MGP (pg/ml)</td>
<td>20.6 (7.6–33)</td>
<td>18.0 (9.2–65.6)</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>(20.3 ± 5.5)</td>
<td>(21.4 ± 10.9)</td>
<td></td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>2.6 (0.4–80)</td>
<td>0.3 (0.1–6.6)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(2.8 ± 1.9)</td>
<td>(0.9 ± 1.5)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± standard deviation (SD) and values of the OPG, MGP and hs-CRP are also expressed as median (range). BMI: body mass index, BP: blood pressure, ALT: alanine aminotransferase, AST: aspartic aminotransferase, OPG: osteoprotegerin, MGP: Matrix Gla protein, hs-CRP: high-sensitivity C-reactive protein.

**Figure 1**  Correlation between osteoprotegerin (OPG) and age in rheumatoid arthritis (RA) patients.

\( r = 0.32, p = 0.02 \) (Fig. 1), but not with MGP concentration \( r = 0.05, p = 0.64 \) in the RA patients. No correlation was found between, OPG and MGP levels and DAS28-CRP in RA patients \( p = 0.4 \) and \( p = 0.8 \) respectively.

### 4. Discussion

Rheumatoid Arthritis is among the most common inflammatory joint disorders, which is associated with systemic inflammatory mediators [34]. Bone destruction is a main unsolved problem in patients with RA. Published data have shown the prevalent role of the OPG/RANK/RANKL triad in bone pathophysiology. RANKL is the main osteoclast-stimulating factor and many clinical studies indicated that increased RANKL and/or decreased OPG, a soluble neutralizing receptor for RANKL, could develop bone erosions. So, bone resorption could be significantly reduced by inhibition of RANK as the receptor of RANKL and also by increasing OPG level [35–37].

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Osteoprotegerin (OPG) and Matrix Gla protein (MGP) in rheumatoid arthritis patients

Diovascular deaths in RA results from accelerated atherosclerosis. The presence and the extent of vascular calcifications are strong predictors for cardiovascular and all-causes of mortality and morbidity in these patients [44–46]. Several factors such as MGP, OPG, Osteopontin (OPN), and Fetuin-A may act as inhibitor mediators for vascular calcification [47,48]. MGP is one major calcification inhibitor, released from hepatocytes. It has been shown that lower serum MGP concentration is independently associated with the risk of cardiovascular and all-cause mortality in patients, especially in renal failure [49,50]. Cranenburg et al. [51] showed that MGP levels are markedly lowered in hemodialysis patients and plasma MGP correlated inversely with coronary artery calcification (CAC) scores in these patients. In our study, we compared the MGP levels in the RA and control subjects and no significant difference was found. Multiple unidentified factors such as genetic polymorphism at the MGP locus may cause such disassociation [52] which should be considered in the future studies.

The correlation of OPG with age in RA patients, in contrast to the healthy controls, was observed in our study. Frasetto et al. [53] in their study have found this relationship also in the healthy populations, which would be due to decreasing GFR with aging. The age-related increase of OPG in our study possibly represents a compensatory mechanism against age-dependent bone loss or the effect of age on vascular calcification in RA patients.

Some weaknesses of our study are lacking of the measurement of the intima-media thickness (IMT), bone density and radiological scores. Also, the possible effects of consumed drugs in RA patients on OPG and MGP as well as hs-CRP levels are major limitations of our study.

In conclusion, the significant elevation of the OPG level in RA patients may through light on its possible role in the pathogenesis of this disease and could be considered as a future therapeutic target. The significant correlation with age suggests that OPG may be an important mediator especially in elderly RA cases. The relationship of OPG and immunoglobulin-FC, and other factors in RA needs further evaluation.

Conflict of interest

None declared.

References


