Correlation between serum osteoprotegerin and atherosclerotic vascular disorders in rheumatoid arthritis patients

Alaa M. Alsalawy, Ahmed I. Fathi, Reda A. Kamel, Ibrahim Ewis

Physical Medicine and Rheumatology Department, Faculty of Medicine, Tanta University, Egypt
National Heart Institute, Cairo, Egypt
Internal Medicine Department, Faculty of Medicine, Zagazig University, Egypt
Clinical Pathology Department, Alazhar University, Egypt

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Abstract  Aim of the work: The aim of the present study was to investigate the association of serum osteoprotegerin (OPG) level with the presence of angiographically documented asymptomatic coronary artery disease (CAD) in patients with rheumatoid arthritis (RA) and to evaluate its relationship with plasma thrombomodulin (TM), as a marker of endothelial dysfunction and with carotid artery intima media thickness (IMT), as a marker of atherosclerosis.

Patients and methods: The study included 20 rheumatoid patients without CAD (negative results on exercise ECG stress test) and other 20 rheumatoid patients with CAD (positive results on exercise ECG stress test and confirmed by coronary angiography). In addition, 20 age and sex matched normal control subjects were studied. Serum OPG and plasma TM levels were measured and carotid artery IMT was determined.
1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by increased mortality largely attributable to cardiovascular disease [1].

Articular bone erosions is a characteristic feature of RA [2] and one of its skeletal complications as well as systemic osteopenia that result in an increased risk of bone fractures [3]. Previous studies investigating the pathogenic mechanisms of bone destruction in animal models of arthritis have provided substantial evidence that the osteoclast is the cell type primarily responsible for focal bone erosion, and its generation and activity are dependent upon receptor activator of Nuclear factor-kB ligand (RANKL) [4,5].

OPG is a newly identified glycoprotein, belonging to the tumor necrosis factor receptor superfamily, originally discovered as an inhibitor of bone resorption. This inhibition is mediated through OPG’s binding and neutralization of RANKL which is a strong inducer of osteoclast differentiation [6]. Interestingly, it has been demonstrated that OPG is produced by a variety of tissues, including the cardiovascular system (heart, arteries, and veins), lung, kidney, and immune tissues, as well as bone [6], and is regulated by various cytokines and hormones [7].

Emerging evidence indicates that osteoporosis and atherosclerotic vascular diseases are commonly found together. Conceptually, a shift of calcium from the skeleton toward the arterial wall can account for both disorders, but the underlying paracrine mechanisms that operate in bone metabolism and vascular homeostasis have not been defined. Mouse genetics may have unraveled the potential molecular link between osteoporosis and arterial calcification [8].

It has been shown that OPG-deficient mice develop both severe osteoporosis and medial calcification of the aorta and renal arteries [9,10], and they were completely prevented by restoration of the gene [11]. Thus, OPG has been proposed as the link between osteoporosis and atherosclerosis. Furthermore, OPG seems to play an important part in preventing erosions and osteoporosis in patients with RA [12]. While in postmenopausal women, a single subcutaneous injection of OPG rapidly reduced biochemical markers of bone resorption [13]. A strong association between serum levels of OPG and severity of coronary artery disease was observed in former research [14]. Moreover, OPG level was found to be higher in rheumatoid arthritis patients [15].

The aim of the present study was therefore to investigate the association of OPG serum level with the presence of angiographically documented asymptomatic CAD in RA patients and to evaluate its relationship with plasma TM as a marker of endothelial dysfunction and with carotid artery IMT as a marker of atherosclerosis.

2. Patients and methods

75 RA patients were collected to be enrolled within the current study with definite RA, diagnosed according to the 2010 American College of Rheumatology (ACR) /European League against Rheumatism (EULAR) classification criteria for rheumatoid arthritis [16]. They were selected from those attending the rheumatology, internal medicine clinics of Mouwasat Hospital, Dammam, KSA. The patients had no history or symptoms of coronary events and with normal resting electrocardiography (ECG). RA patients were further recruited according to the presence or absence of CAD (28 patients without CAD, 21 patients with CAD, and 26 patients refused to continue with the procedures of the research so they had been excluded from the study) into two groups, Group I: included 20 rheumatoid patients (selected from originally 28 patients) without CAD (negative results on exercise ECG stress test) and Group II: included 20 rheumatoid patients (selected from originally 21 patients) with CAD (positive results on exercise ECG stress test and confirmed by coronary angiography).

A written consent was taken from all enrolled patients before starting any of the procedures of the research.

Patients suffering from uncontrolled hypertension, acute infection, renal disease (serum creatinine > 2.0 mg/dl), diabetes mellitus, or malignancies were excluded from the study.

Twenty volunteers of matched age (50.2 ± 3.3) and sex (14 females and 6 males), normotensive, 12 were smokers (60%), with vertebral Bone Mineral Density (BMD) T-score was −1.1 ± 0.9, and hip BMD T-score was −0.5 ± 0.8 with normal ECG (resting and exercise) and carotid duplex findings had been participated as control subjects.

All cases included in this study were subjected to the following:

- Complete history taking and clinical examination with special stress on cardiac symptoms, presence of cardiovascular risk factors such as smoking, hypertension, dyslipidemia, family history of premature CAD. Body mass index (BMI), duration of rheumatoid, menopausal history (all females patients included in the study were on perimenopausal stage i.e. irregular menses in the last
15 months, and treatment undertaking (all RA patients were under Methotrexate as a monotherapy disease modifying antirheumatic drugs [DMARDs] in a dose between 12.5 and 15 mg/week, and consuming non-steroidal anti inflammatory drugs [NSAIDs] on irregular basis, stopped corticosteroid therapy in last 5 years of the disease duration and on daily requirements of Calcium and Vitamin D).

- Disease activity in RA patients was assessed by measuring DAS-28 [17] and the Modified Health Assessment Questionnaire (MHAQ), a standard 8-question instrument, was used to assess functional capacity based on the difficulty in performing activities of daily living [18].

- Laboratory investigations including serum lipid profile, ESR, CRP, serum blood glucose, kidney function tests and BMD T-scores were documented from patient’s files on their regular visits, while plasma TM and serum OPG were measured.

- Resting and exercise ECG stress test.

- Coronary angiography in patients with positive results on exercise ECG stress test.

- Carotid IMT measurement.

Figure 1  Coronary angiography showing significant left coronary artery disease (narrowing and turtuosity).

Figure 2  Coronary angiography showing significant right coronary artery disease (occlusion).
2.1. Laboratory procedures

Venous blood samples were collected from patients and controls under aseptic precautions, serum and citrated plasma were prepared from the fasting blood sample, aliquoted and stored at −20 °C until further analysis.

2.2. Estimation of plasma TM

Plasma TM was quantified by ELISA technique using a two site monoclonal enzyme immunosorbent assay for TM provided by Diagnostica Stago Asserachrom, Asmieres, France as described in [19].

2.3. Determination of serum OPG

Serum OPG was measured by a commercially available kit (R&D Systems, Minneapolis, MN, USA) as described in [20].

2.4. Exercise ECG stress test

Antihypertensive treatments with B-adrenergic blocking agents together with food and beverages containing caffeine asked to stop for at least 12 hours before the test. An exercise test was performed according to the Bruce protocol using a Cardiocontrol treadmill [21].

2.5. Coronary angiography

In patients with positive results on exercise ECG stress test, diagnostic coronary angiography was performed using the standard Judkin’s technique on Philips catheter system and all images were digitally stored in Dicom format on a Hewlett Packard visualize work station for further analysis. A narrowing of > 50% in luminal diameter for one of the major epicardial coronary arteries was considered hemodynamically significant, and the patient was classified as having CAD [21], Figs. 1 and 2.

2.6. Carotid intima media thickness

Carotid duplex scanning was performed on all subjects who participated in the study. Ultrasound examination was done using a 7.5 MHz linear-phased array transducer and imaging system of Hewlett Packard Sonos 5500. Right and left distal common carotid arteries were examined following standard procedures as in [22], values less than 0.8 mm correlate with lack of CAD whereas an increasing thickness above was considered abnormal [22,23].

Statistics: Results were expressed as mean ± SD. Comparisons between groups were made using Student’s t-test for continuous variables. Correlation between the two parameters was determined by Pearson’s correlation coefficient (r). Odds ratios (ORs) were estimated with corresponding 95% confidence intervals (CIs). P values < 0.01 were considered statistically significant.

3. Results

The demographic, clinical and laboratory features of rheumatoid arthritis patients with and without CAD are presented in Table 1. Group I included 16 females and 4 males, their mean age was 52.4 ± 3.2 years, the mean duration of disease was 8.3 ± 2.2 years, their mean body mass was 27.2 ± 1.9, and 16 patients (80%) had rheumatoid factor (RF) positive (above 20 iu/ml) while 19 patients (95%) had anticitrullinated protein antibodies (ACPA) positive. Group II included 17 females and

<table>
<thead>
<tr>
<th>Variables</th>
<th>Rheumatoid without CAD (n = 20)</th>
<th>Rheumatoid with CAD (n = 20)</th>
<th>Control (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.43 ± 3.2</td>
<td>51.1 ± 3.8</td>
<td>50.2 ± 3.3</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>16/4</td>
<td>17/3</td>
<td>14/6</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.2 ± 1.9</td>
<td>29.6 ± 2.1</td>
<td>26 ± 1.6</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>5 (25%)</td>
<td>14 (70%)</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>8.3 ± 2.2</td>
<td>10.2 ± 4.2</td>
<td>–</td>
</tr>
<tr>
<td>Hypertensive n (%)</td>
<td>7 (35%)</td>
<td>12 (60%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Positive RF n (%)</td>
<td>16 (80%)</td>
<td>18 (90%)</td>
<td>–</td>
</tr>
<tr>
<td>Positive ACPA n (%)</td>
<td>19 (95%)</td>
<td>20 (100%)</td>
<td>–</td>
</tr>
<tr>
<td>Family history of CAD, n (%)</td>
<td>2 (10%)</td>
<td>4 (20%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>235 ± 17</td>
<td>267 ± 30</td>
<td>187 ± 12</td>
</tr>
<tr>
<td>Serum triglycerides (mg/dl)</td>
<td>160 ± 20</td>
<td>180 ± 32</td>
<td>145 ± 24</td>
</tr>
<tr>
<td>Serum HDL-C (mg/dl)</td>
<td>35 ± 1.7</td>
<td>33 ± 1.4</td>
<td>59 ± 90</td>
</tr>
<tr>
<td>Serum LDL-C (mg/dl)</td>
<td>147 ± 24.5</td>
<td>170 ± 35</td>
<td>118 ± 29</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>40.80 ± 10.7</td>
<td>40.75 ± 9.22</td>
<td>–</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>20.5 ± 10.7</td>
<td>22.8 ± 9.7</td>
<td>–</td>
</tr>
<tr>
<td>BMD T-score</td>
<td></td>
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</tr>
<tr>
<td>(i) Vertebral</td>
<td>−1.8 ± 0.8</td>
<td>−1.7 ± 0.6</td>
<td>−1.1 ± 0.9</td>
</tr>
<tr>
<td>(ii) Hip</td>
<td>−0.9 ± 0.9</td>
<td>−1.1 ± 0.3</td>
<td>−0.5 ± 0.8</td>
</tr>
<tr>
<td>MHAQ</td>
<td>2.25 ± 0.847</td>
<td>2.38 ± 0.72</td>
<td>–</td>
</tr>
<tr>
<td>DAS28</td>
<td>4.35 ± 0.8</td>
<td>4.26 ± 0.63</td>
<td>–</td>
</tr>
</tbody>
</table>

Rheumatoid factor (RF), Anti-citrullinated protein antibodies (ACPA), High density protein (HDL), Low density protein (LDL), Erythrocytes sedimentation rate (ESR), C reactive protein (CRP) and Bone mineral density (BMD).
3 males, their mean age was 51.1 ± 3.8 years, mean duration of the disease is 10.2 ± 4.2 years, their mean body mass index was 29.6 ± 2.1, and 18 patients (90%) had rheumatoid factor (RF) positive (above 20 IU/ML) while all of them 20 patients (100%) had anticitrullinated protein antibodies (ACPA) positive.

Serum OPG level and plasma TM were significantly higher in RA patients with CAD than in those without CAD and controls (P < 0.001), also OPG level in RA patients without CAD as compared to controls (P < 0.01), however, there was no significant difference in plasma TM between patients without CAD and controls as shown in Table 2. Carotid artery IMT was significantly increased in RA patients with and without CAD as compared to controls (P < 0.01) (2) Table 3.

In RA patients without CAD, serum OPG correlated significantly with age (r = 0.69, P < 0.001), duration of disease (r = 0.71, P = 0.001), carotid IMT (r = 0.65, P < 0.01), DAS-28, ESR and CRP whereas there were no correlations between serum OPG and BMI, serum lipid profile, plasma TM, or MHAQ (3). In those with CAD, serum OPG correlated significantly with age, duration of rheumatoid, plasma TM, DAS-28, ESR, CRP, MHAQ and carotid IMT (P < 0.001) but not with BMI or serum lipid profile (3).

Figs. 1 and 2 showed changes that occurred in left and right coronaries in patients with CAD (group II) which ranged between narrowing and tortuosity as in Fig. 1, and complete occlusion as in Fig. 2.

4. Discussion

Chronic inflammation plays an important role in pathogenesis of atherosclerosis and RA. There is increasing evidence from controlled clinical studies stating that there is a high incidence of cardiovascular diseases in RA [24–26].

It has been documented that progression of atherosclerotic calcification is inversely correlated with low bone tissue mineralization [27] and is associated with bone loss in older men [28] and in postmenopausal women [29,30]. Jesper et al [27] documented that loss of bone mass in osteoporosis leads to increased circulating phosphate and calcium and decreased parathyroid hormone, which stimulate mineralization of cardiovascular tissue, also pointed on the importance of cytokines parathyroid hormone, which stimulate mineralization of cardiovascular tissue, also pointed on the importance of cytokines.

Importantly, OPG levels provide prognostic information in patients who develop heart failure after acute myocardial infarction (AMI) [33], so OPG might represent a novel marker of plaque instability and cardiovascular mortality in these patients [34].

Since a strong association has been reported between OPG and the presence and severity of CAD [35,36], this study was designed to investigate the association of OPG serum levels with the presence of asymptomatic CAD in patients with rheumatoid arthritis and to evaluate its relationship with plasma TM as a marker of endothelial dysfunction and with carotid IMT as a marker of atherosclerosis.

Serum levels of OPG were significantly higher in RA patients with and without CAD compared to controls (P < 0.001 and P < 0.01; respectively). There were also significant positive correlations between OPG serum levels and age, duration of rheumatoid arthritis, ESR, CRP and DAS-28 in patients with and without CAD however, no correlations were found between serum OPG and BMI and any of lipid profiles.

Although the function of OPG in the vasculature is debatable, our results were compatible with the findings of Shopped et al, [14] who described increased plasma concentrations of OPG in men with CAD also with the results of work of Unna et al, [37] who could explain the important role of RANKL/OPG system in pathogenesis of atherosclerosis in inflammatory rheumatic diseases.

Solomon et al, [38] demonstrated that several inflammatory biomarkers linked to cardiovascular diseases (CVD) were significantly elevated in women with RA, including CRP, fibrinogen, serum intercellular adhesive molecules (sICAM-1), soluble tumor necrosis factor receptor I and II (stoner I&II), and OPG. The elevation of OPG in the context of RA has been reported by previous published study which explained that OPG is modulated by the immune system, and may act as a counter-regulatory molecule that compensates for increased production of RANK [25].

The positive associations between OPG concentrations and ESR, CRP and duration and activity of RA observed by Kubota et al [39] and Ziolkowska et al [15] who reported high-

### Table 2 Comparison of serum OPG, plasma TM and carotid IMT in patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Serum OPG (pmol/l)</th>
<th>Plasma TM (ng/ml)</th>
<th>Carotid IMT (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 20)</td>
<td>1.6 ± 1.2</td>
<td>37 ± 6.6</td>
<td>0.59 ± 0.19</td>
</tr>
<tr>
<td>Rheumatoid without CAD (n = 20)</td>
<td>3.1 ± 1.7</td>
<td>42.1 ± 10.5</td>
<td>0.81 ± 0.23</td>
</tr>
<tr>
<td>Rheumatoid with CAD (n = 20)</td>
<td>6.3 ± 2.8</td>
<td>77.8 ± 15.4</td>
<td>1.36 ± 0.28</td>
</tr>
</tbody>
</table>

P1 rheumatoid without CAD vs. controls.
P2 rheumatoid with CAD vs. controls.
P3 rheumatoid with vs. without CAD.
* P < 0.01 significant.
er serum levels of OPG in RA patients and documented that proinflammatory cytokines enhanced OPG production and anti-TNF alpha treatment resulted in the normalization of serum OPG levels in RA patients. In addition to its association with IMT of carotid arteries in healthy postmenopausal women [41] and also in end stage renal disease [42].

The results of our study showed that carotid artery IMT was increased in rheumatoid patients with and without CAD as compared to controls (P < 0.001 and P < 0.01; respectively) and serum OPG correlated significantly with IMT in rheumatoid patients with and without CAD (P < 0.001 and P < 0.01; respectively). This finding is in agreement with Kiechl et al. (2004) [20], who found that OPG was significantly and independently related to severity and 10-year progression of carotid atherosclerosis. Golledge et al. [40] also reported increased concentrations of OPG within unstable (symptomatic) compared with stable (asymptomatic) carotid atherosclerosis. Moreover, serum OPG level was positively correlated with IMT of carotid arteries in healthy postmenopausal women [41] and also in end stage renal disease [42].

We also observed that serum OPG level was correlated with plasma thrombomodulin (TM) a marker of endothelial dysfunction, only in rheumatoid patients with CAD, but not in those without CAD as compared to controls. These findings are correlated with Chung et al. [1] and Roman et al. [24] who described in their studies that patients with RA have accelerated atherosclerosis and their findings suggested that serum OPG is a better and early predictor of atherosclerosis in rheumatoid arthritis.

Although the precise biological role of OPG in vascular diseases remains unclear, elevated serum levels of this vascular protective factor could be interpreted as an insufficient compensatory self-defensive mechanism against factors that promote arterial calcification and atherosclerosis [43]. Therefore, it could be assumed that raised OPG is a response to rather than a cause of atherosclerosis, in an attempt to prevent further vascular damage which correlate with the findings of Shaker et al. [2] who stated in their study that elevation of OPG levels may represent a crucial compensatory mechanism to limit further vascular damage. Moreover, Pedersen et al. [44] demonstrated that elevated OPG levels are associated with increased risk of acute events in stable angina pectoris patients, though independent effects are restricted to a particular subgroup with markedly enhanced activity in the OPG/RANKL/RANK system, as reflected by high OPG levels. However, at high concentrations, OPG can also enhance the matrix metalloproteinase (MMP)-inducing effect of RANKL as well as having metalloproteinase (MMP)-inducing and chemotactic effects of its own [45,46]. Serum OPG level therefore may reflect inflammation and matrix degradation as well as vascular calcification.

Thus, therapeutic strategies aimed at increasing local OPG concentrations in bone and the vascular wall using gene therapy with human recombinant OPG, might be protective against osteoporosis and atherosclerosis [47].

By investigating OPG and other bone-related molecules in relation to the arterial wall, we would gain a new perspective on the understanding of the molecular mechanisms underlying the development of macrovascular disease in rheumatoid arthritis.

In conclusion, from this study, it could be concluded that osteoprotegerin is a clinically important molecule, independently associated with the presence of coronary artery disease and may be a good indicator of atherosclerotic vascular damage (macroangiopathy) in rheumatoid arthritis. Hence, measurement of serum OPG merits further investigation as a simple test for improving early diagnosis of asymptomatic CAD in rheumatoid arthritis patients. Further larger, prospective studies are required to clarify the causative relation between OPG, rheumatoid arthritis and atherosclerotic vascular disease as well as clinical trials of novel agents for correcting causal mechanisms and to evaluate a possible therapeutic potential of OPG as a new “vasculoprotegerin”.

Future research on OPG gene polymorphisms could play a role in the development, progression and response to therapy in rheumatoid vascular complications.

References


