Circulating Matrix Metalloproteinase-9 Concentrations Do Not Indicate Ongoing Inflammation and Accelerated Atherosclerosis in Rheumatoid Arthritis

Plasma Matriks Metalloproteinaz-9 Düzeyleri Romatoid Artritte Devam Eden İnflamasyon ve Hızlanmış Aterosklerozun Bir Göstergesi Değildir

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**ABSTRACT Objective:** The aim of the present study was to evaluate the potential roles of matrix metalloproteinase-9 in the development of accelerated atherogenesis and active systemic inflammation in rheumatoid arthritis patients. **Material and Methods:** Forty-seven rheumatoid arthritis patients (38 women, 9 men) and 20 healthy controls (15 women, 5 men) were included. Rheumatoid arthritis patients were divided into two subgroup as active (20 women and 4 men) and inactive (18 women and 5 men) disease. Enzyme-linked immunosorbent assay kit with monoclonal antibodies against the substance was used to measure plasma matrix metalloproteinase-9 levels. Carotid intima thickness of the subjects were measured by high resolution B-mode ultrasound and a average-intima media thickness (right plus left intima media thickness divided by 2) value was calculated for each subject. **Results:** Inactive and active rheumatoid arthritis patients had significantly higher median average-intima media thickness compared to control group (p= 0.003 and p= 0.001, respectively). Median average-intima media thickness did not differ between active and inactive rheumatoid arthritis patients. Median matrix metalloproteinase-9 levels did not differ between studied groups. There was no correlation between matrix metalloproteinase-9 levels and average-intima media thickness. Matrix metalloproteinase-9 did not correlate with erythrocyte sedimentation rate, C-reactive protein and disease activity score 28. **Conclusion:** Rheumatoid arthritis is associated with increased carotid intima media thickness which is a sign of accelerated atherosclerosis however, findings of the present study does not suggest a role for matrix metalloproteinase-9 in this process.

**Key Words:** Matrix metalloproteinase 9; arthritis, rheumatoid

**ÖZET Amaç:** Romatoid artritte hastalarında aktif sistemik inflamasyon ve hızlanmış ateroskleroz gelişiminde matriks metalloproteinaz-9 (MMP-9)‘un potansiyel rolünün incelenmesi. **Gereç ve Yöntemler:** Kirk yedi (38 kadın, 9 erkek) romatoid artrit hastası ve 20 (15 kadın, 5 erkek) sağlıklı control grubu dahil edildi. Romatoid artrit hastaları aktif (20 kadın, 4 erkek) ve inaktif (18 kadın, 5 erkek) olarak 2 alt gruba ayrıldı. Plazma MMP-9 düzeyleri MMP-9 ELISA kiti kullanılarak ölçüldü. Karotis intima-media kalınlığı yüksek çözünürlüklü B-mode ultrasonografi cihazı kullanılarak ölçüldü. Sağ ve sol karotis arterler ait ölçümler toplanarak 2’ye bölündü ve her bir hasta için ortalama değerler bulundu. **Bulgular:** Aktif ve inaktif romatoid artrit hastaları kontrol grubu ile karşılaştırıldığında anlamli düzeyde daha yüksek median intima medi-kalınlıklarına sahiplerdi (p değerleri sırası ile: 0.003 ve 0.001). Aktif ve inaktif romatoid artrit hastalarında ise medyan intima media kalınlıkları arasında anlamli farklı yoktu. Her üç grupta da medyan MMP-9 seviyeleri benzerdi. MMP-9 seviyeleri ile intima media kalınlığı, eritrosit sedimentasyon hızı, C-reactif protein ve romatoid artrit hastalığın aktif vitesi arasında anlamli bir korelasyon saptanmadı. **Sonuç:** Romatoid artrit, hızlanmış aterosklerozun bir işareti olan artmış karotis intima media kalınlığı ile ilişkili bir hastalık ol maksıla beraber, MMP-9’un bu olayda herhangi bir rolünden söz edilememektedir.

**Anahtar Kelimeler:** Matriks metalloproteinaz 9; artrit, romatoid

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rheumatoid arthritis (RA) is a chronic systemic autoimmune rheumatic disorder, characterized by inflammation, synovial hyperplasia and destruction of the affected joints. The cellular activation of aggressively growing, matrix-degrading synovial fibroblasts is a prominent feature in the pathobiology of RA. Joint destruction in RA is mediated by the attacks of numerous enzymes such as serine proteases, cathepsins, and the matrix metalloproteinases (MMPs). MMPs are a family of enzymes that degrade different components of extracellular matrix. Although MMPs play an important role in normal physiologic processes such as maintaining tissue remodeling and regeneration, their excessive expression degrade non-collagen matrix components of the joints as in RA.

Atherosclerosis is an ongoing long-term pathological process associated with the inflammation in the course of RA. Patients with RA have about four times increased risk of developing premature cardiovascular diseases, which could occur even in the absence of the classical risk factors associated with the atherosclerosis. Human carotid atherosclerotic plaques are rich in foamy macrophages, which are also found in the lining layers and sublining areas of RA synovium. The macrophages are involved in the induction of MMP-9 (Gelatinase B) which contribute to cartilage destruction in RA and extracellular matrix degradation in atherosclerosis. The aim of the present study was to evaluate the potential implication of MMP-9 in the development of accelerated atherogenesis and active systemic inflammation in RA patients.

MATERIAL AND METHODS

PATIENTS AND CONTROLS

Fourty-seven RA patients (38 women, 9 men), and 20 healthy controls (15 women, 5 men) were included in the study. All RA patients fulfilled the 1987 American Rheumatism Association criteria. All subjects were younger than 60 years at the time of the enrollment. In the RA patients, disease activity was assessed by disease activity score (DAS28) and the score ≤ 3.2 was considered inactive disease. Thus, RA patients were divided in to two subgroups as active and inactive RA. 24 patients (20 women, 4 men) had active RA and 23 patients (18 women, 5 men) had inactive RA.

The exclusion criteria included; age older than 60 years, malignancy, chronic renal failure, diabetes mellitus, impaired fasting glucose, impaired glucose tolerance (fasting glucose ≥100 mg/dL and OGGT second hour glucose ≥140 mg/dL), history of cerebrovascular disease, peripheral arterial disease, coronary arterial disease and uncontrolled arterial hypertension (systolic pressure > 140 mmHg and/or diastolic pressure > 90 mmHg).

The Ethics Committee of Gazi University Faculty of School approved the study. Written informed consent was obtained from all subjects.

MEASUREMENT OF PLASMA MMP CONCENTRATION

Blood samples were taken from peripheral veins after an overnight fast and collected in ice-cold vacuum glass tubes containing EDTA. Then, the plasma was separated by centrifugation at 3000 rpm for 10 min at 4°C. These samples were immediately frozen and stored at -80°C. Enzyme-linked immunosorbent assay (ELISA) kit with monoclonal antibodies against the substance (RayBio® Human MMP-9 ELISA Kit) was used to measure plasma MMP-9 levels according to the instructions provided by the manufacturer. The sensitivity of ELISA for MMP-9 was 0.001 ng/mL. The MMP-9 assay was done in duplicate and the mean intra-assay coefficient of variation and mean interassay coefficient of variation for both the assays were 10% and 12%, respectively.

VASCULAR DISEASE MEASUREMENTS

Carotid intima media thickening (IMT) of the subjects were measured by high resolution B-mode US by a single experienced examiner who was blinded to the clinical and biochemical data. All patients were evaluated by high-resolution ultrasound using the Logiq 9 system (GE Medical Systems, Milwaukee, WI) and a 7.5 MHz linear array transducer. IMT of the right and left common carotid arteries were measured within the 1 cm segment.
proximal to the dilatation of the carotid bulb at sagittal planes. Patients and controls were examined in supine position with the neck extended and the chin turned contralateral to the side being examined. IMT was measured in the right and left common carotid artery at the level of carotid bifurcation in the posterior wall, and the means of both values were calculated. All measurements were made manually on digitized still images that were obtained during ultrasound scanning. The carotid plaques were excluded while measuring IMT. A average IMT (right plus left IMT divided by 2) value was calculated for each subject.

STATISTICAL ANALYSIS

Data are expressed as median (interquartile range = IQR). Quantitative variable were compared using Kruskal Wallis test. If p value was less than 0.05, we used the Mann-Whitney U test on each pair of groups and adjusted the p value with the Bonferroni. Therefore, a p value of less than 0.016 was considered statistically significant. Spearman correlation coefficient was used for correlation analysis.

RESULTS

Characteristics of the patients and controls are depicted in Table 1. Age, sex, body mass index (BMI) and risk factors for atherosclerosis were similar between groups. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were highest in the active RA, and inactive RA had higher values compared to controls. In paired comparisons, only active RA group compared with both inactive RA and control groups reached statistical significance. All lipid parameters except for low density lipoprotein (LDL) were similar in all three groups. LDL levels were the highest in the controls, and active RA had higher level compared to inactive RA. There was significant difference only between inactive RA and control groups. Fibroinogen and homocysteine levels were similar in all three groups. P values from overall and paired comparisons are given in Table 1.

| TABLE 1: Characteristics of patient and control groups. |
|---------------------------------|-----|-----|-----|-----|
| **Median (IQR)**                |     |     |     |     |
| Disease duration (years)        | 5(2-7) | 4(1-6) | - | 0.242 |
| Sex (Female/male)               | 20/4 | 18/5 | 15/5 | 0.724 |
| Age (year)                      | 50 (41-55) | 51 (37-58) | 46 (42-52) | 0.445 |
| BMI (kg/m²)                     | 26 (24-27.7) | 27 (25-30) | 27 (25-28) | 0.522 |
| ESR (mm/hour)                   | 31 (24-40) | 21 (12-26) | 12 (9-19) | < 0.001* |
| CRP (mg/dL)                     | 5 (0-27) | Negative | Negative | - |
| DAS28                            | 4 (3.5-4.58) | 2.3 (2.08-2.68) | - | < 0.001 |
| Fibroinogen (mg/dL)             | 387 (292-424) | 305 (288-345) | - | 0.054 |
| Total cholesterol (mg/dL)       | 190 (179-214) | 182 (165-199) | 207 (171-248) | 0.132 |
| LDL (mg/dL)                     | 117 (102-136) | 99 (89-123) | 137 (102-153) | 0.034† |
| HDL (mg/dL)                     | 48 (44-55) | 55 (47-64) | 47 (43-57) | 0.118 |
| Triglyceride (mg/dL)            | 121 (100-143) | 122 (90-137) | 101 (80-151) | 0.784 |
| Lp (a) (mg/dL)                  | 16.1 (9.9-24.2) | 16.8 (9.9-22.2) | 16 (10.2-24.2) | 0.922 |
| Homocysteine (µmol/L)           | 8.7 (7.4-11.8) | 8.9 (7.5-12.2) | 11.6 (8.6-14.6) | 0.170 |
| Smoking                         | 6 (%25) | 5 (%21.7) | 6 (%30) | 0.824 |
| Family history                  | 3 (%12.5) | 6 (%26.1) | 5 (%25) | 0.286 |
| Hypertension                    | 5 (%20.8) | 7 (%30.4) | 5 (%25) | 0.751 |
| Hyperlipidemia                  | 7 (%29.2) | 4 (%17.4) | 5 (%25) | 0.633 |
| RF positivity                   | 14 (%58.3) | 15 (%65.2) | - | 0.631 |

*Active RA vs inactive RA and active RA vs control (each two p values < 0.001 for ESR and < 0.001 and = 0.014 respectively for CRP), † Inactive RA vs control (p< 0.012).

BMI: Body mass index, ESR: Erythrocyte sedimentation index, CRP: C-reactive protein, LDL: Low density lipoprotein, HDL: High density lipoprotein.
Inactive RA patients had the highest median average-IMT values, and active RA patients had higher values compared to controls (0.81 (0.71-0.94), 0.78 (0.70-0.97), 0.61 (0.53-0.78) respectively; overall p= 0.002). In paired comparisons, although both RA groups had significantly higher median average-IMT compared to control group, the difference between active and inactive RA groups was not statistically significant (Figure 1A). Median MMP-9 levels did not differ between groups (2.87 (1.54-3.10), 3.01 (1.14-3.08), 3.06 (1.98-3.23), respectively; overall p= 0.24) (Figure 1B).

There was no correlation between MMP-9 level and average-IMT when all subjects were included in the analysis (r= 0.011, p= 0.93) as well as when only RA patients were analyzed (r= 0.153, p= 0.30). In addition, we did not find any association between MMP-9 level and ESR (r= 0.078 p= 0.60), CRP (r= 0.096, p= 0.052) or DAS28 (r= 0.002, p= 0.98).

**DISCUSSION**

Rheumatoid arthritis, active or inactive, is associated with increased carotid intima-media thickening which is a sign of accelerated atherosclerosis. Findings of the study does not suggest a role for MMP-9 in this process. Roman and coworkers recently demonstrated that patients with RA, despite a more favorable classical cardiovascular risk profile, had a three-fold increase in carotid atherosclerotic plaque. The relationship between RA and carotid atherosclerotic plaque remained significant after accounting for age, serum cholesterol levels, smoking history, and hypertensive status in their study. Our results are comparable and support the hypotheses that the clinical course of RA is associated with preclinical atherosclerosis, independent of known cardiovascular risk factors, and disease severity associated with chronic inflammation are atherogenic in RA. In addition, our results demonstrated that serum LDL levels were significantly lower in inactive RA patients than controls, while serum LDL levels were comparable in active RA patients and controls. In the medical literature, some studies showed significant elevation in serum LDL levels when compared to controls, but some others did not. Two different studies from Turkey demonstrated that serum LDL levels were similar between RA patients and healthy subjects. Initial lipid profile in active RA patients improved after successful therapy. Hence, our results might be explained by the effect of successful RA therapy in the lipid profile of active RA patients. Furthermore, since inactive RA patients had also higher carotid-IMT than controls despite lower serum LDL levels, our result supported previous studies suggesting that RA patients had an accelerated atherosclerosis independent of classical risk factors for cardiovascular disease.

The results of the present study disclosed that serum levels of MMP-9 cannot be used as a reliable predictive factor to assess disease severity, active inflammation, or accelerated atherosclerosis of RA. An ideal “surrogate marker of disease” should reflect ongoing active inflammation and atherosclerosis even in patients receiving disease-modifying drugs. MMPs are locally expressed key regulators in the pathobiological basis of RA. Once critically controlled cell migration with MMPs is lost, inflammatory cell migration facilitates disease progression in RA. However, the basic functions of MMPs mainly take place in the local RA synovium and other extraarticular local microenvironments. Several previous studies proposed that circulating levels of MMPs may reflect the disease activity of RA. However, MMP levels are affected from the disease-modifying drugs of RA, such as NSAIDs and TNF blockers. Disease course of RA and atherosclerotic process may exhibit alterations in distinct populations. These reasons might explain the absence of any association with serum MMP-9 le-
levels and disease severity, active inflammation, or accelerated atherosclerosis in this study.

The association of RA with accelerated atherosclerosis may be interrelated with the systemic characteristic inflammatory load of RA and the accumulation of classical cardiovascular risk factors. There are limited data regarding the benefits of cardiovascular risk reduction therapies in RA. Endothelial injury/dysfunction, an early step in the atherogenesis, is observed in RA. Increased carotid intima-media thickness and carotid plaques via a high-resolution B-mode ultrasound studies of carotid arteries have shown the presence of subclinical atherosclerosis in RA. Inflammation affects the mechanisms of vulnerable plaque in atherosclerosis plaque. Since RA is a systemic inflammatory status, the involvement of inflammatory mediators, in connection with proatherosclerotic factors and endothelial dysfunction in the development of atherosclerosis in RA should be searched. Further clinical and experimental studies are required to detect missing links in the cardiovascular risk stratification algorithms and complicated inflammatory course of RA.

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**REFERENCES**

