THE FREQUENCY OF OMENTIN VAL109ASP POLYMORPHISM AND THE SERUM LEVEL OF OMENTIN IN PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT
Rheumatoid arthritis (RA) is an inflammatory and autoimmune disease, affecting approximately 1% population. It is characterized by irreversible destruction of articular cartilage. Omentin has been recently identified as involved in several inflammatory diseases. The aim of this study is to evaluate the potential role of omentin in pathogenesis of RA both genetic and protein levels.

87 patients who applied to Düzce University School of Medicine, Physical Medicine and Rehabilitation Clinic were included in this study. Serum omentin level and Val109Asp genotypes were determined by Enzyme - Linked Immuno Sorbent Assay (ELISA) and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) methods respectively.

There was no significant difference between patient and control groups in terms of serum omentin level and Val109Asp polymorphism.

This study is unique cause is the first study to investigate the possible role of omentin adipokine in the pathogenesis of RA in Turkish population. There was no significant relationship between omentin and RA disease. Our patients had been diagnosed previously and they started to use medicine. This can be considered to be a major limitation of our study. However, to elucidate the putative role of the omentin in the pathogenesis of RA, this study should be conducted on a larger population with more appropriate subjects.

Key words: Omentin, Rheumatoid Arthritis, Polymorphism, ELISA.

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Introduction
Cartilage, an important component of human organism, is mainly composed of chondrocytes and extracellular matrix (ECM) components. It is characterized by small number of chondrocytes which are responsible for the production of ECM components such as collagens and proteoglycans. Among the extraordinary features of cartilage are the distribution of the load and minimizing peak stresses through high water content, which is tightly held within a matrix of negatively charged macromolecular complexes. Under certain pathological conditions, cartilage-specific molecules may become a target of unregulated immune response and trigger a chronic inflammatory process causing joint injury like rheumatoid arthritis[1-3].

Rheumatoid arthritis (RA), an inflammatory and autoimmune disease, is best characterized by irreversible destruction of articular cartilage. While RA affects approximately 1% of the world population, it is 2 or 3 times more common in women than in men, and its prevalence reaches a peak between the ages of 45 and 54. RA is a systemic disease in that it affects not only joint but also internal organs such as heart and skin[4-7]. Its causes have not been clarified in detail yet, but it is thought that genetics, environment and autoimmunity interfere with the mechanisms of RA. Even though there are considerable numbers of studies relating to gene polymorphism in literature, the impact of the genetic factor in the pathogenesis of RA remains a mystery for most polymorphisms which might have potential predisposition to RA. Therefore, it was reported to
need further investigations\textsuperscript{(8, 9)}.

The adipokines (adipocytokines) derived from adipocytes have paracrine, autocrine and even endocrine effects. Up to date, several adipokines such as apelin, resistin, adiponectin, visfatin and omentin have been characterized since the cloning of leptin in 1994, the first characterized adipokines. Today it is possible to realize that the adipose tissue is able to secrete several soluble factors such as chemokines, adipokines etc. like an endocrine organ and these adipokines are involved in the development and progression of some rheumatic diseases like rheumatoid arthritis\textsuperscript{(10-11)}. Among the adipokines, omentin has recently been discovered and located in the first chromosome with 8 exons and 7 introns\textsuperscript{(12, 13)}. The involvement of omentin in many conditions such as coronary artery disease, end-stage renal disease etc. has been shown\textsuperscript{(14, 15)}. The putative involvement of the omentin like other adipokines in the pathogenesis of RA has not been investigated in detail up to date. The aim of this study is to evaluate the potential role of omentin in pathogenesis of RA in both genetic and protein levels.

Material and methods

\textbf{Study Subjects}

This study was conducted over 87 patients who applied Düzce University School of Medicine, Physical Medicine and Rehabilitation (PMR) Clinic. The patient group consisted of 45 cases, diagnosed with RA according to the revised criteria of American College of Radiology (ACR 1987) in 1987. The control group consisted of 42 cases who applied to the PMR Clinic due to various complaints other than inflammatory reasons. The local ethic committee approval was previously obtained (Ethic certificate number: 2012-251). The subjects participating in the study were informed about methodology, expected benefits and potential hazards of this study and written consent was obtained from them according to the Declaration of Helsinki.

The patients with endocrine, systematic and metabolic diseases (such as diabetes mellitus, hypothyroidism, hyperthyroidism, hyperparathyroidism etc.), inflammatory and other muscle diseases (such as polymyositis, dermatomyositis etc.), neuromuscular and neurological diseases, and other rheumatic diseases were excluded.

The demographic features (age, gender, height, body weight and occupation) of the subjects were recorded. The belly and hip circumference of the individuals were measured. The history of disease was recorded in detail, and systemic and locomotor system examinations were conducted. Moreover, in addition to the disease duration and drugs used by RA patients, the laboratory results (complete blood cell count, Erythrocyte Sedimentasyon Rate (ESR), C-reactive Protein (CRP), Rheumatoid Factor (RF), lipid profile, fasting blood glucose, insulin and hemoglobin A1C) of individuals were also recorded and evaluated.

\textbf{DNA extraction and genotyping Omentin Val109Asp Polymorphism}

The genotyping of Omentin Val109Asp Polymorphism was determined as explained before\textsuperscript{(16, 17)}. Briefly, blood samples were obtained from each individual into 2 mL tubes with Ethylenediaminetetraacetic acid (EDTA). Genomic DNA was obtained from peripheral blood leukocytes using a nucleic acid extraction kit (Vivantis, Malaysia), and the samples were stored at -20 °C until analysis. The genotyping was performed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers (F: 5’-GAGCCTTTAGGCCATGTCTCT-3’, and R: 5’-CTCTCCTTCTTCTCAGCCCAT-3’) were used for amplification of 471 bp DNA fragment containing Val109Asp polymorphism. PCR was performed under following conditions; 5 min at 94 oC followed by 35 cycles of 1 min at 94 oC, 1 min at 58 oC, and 1 min at 72 oC, with a final step at 72 oC for 10 min. The amplicon was digested with 10U Xmil (AccI) restriction enzyme (NEB, UK) at 37 oC overnight. The digested products were separated on a 2% agarose gel stained with ethidium bromide. The Asp/Asp genotype has no digestion site. However, the Val/Val genotype generates two bands with 274 and 197 bp sizes and Val/Asp genotype generates three bands with 471, 274 and 197 bp sizes.

\textbf{Serum omentin level analysis}

Serum Omentin level was analyzed using commercially available Omentin-1 enzyme-linked immunosorbent assay (ELISA) kit (BioVendor, Modrice, Czech Republic) according to manufacturer instruction.
Statistical Analysis
Statistical analyses were performed using PASW 18 statistical software (ver. 18.0 for Windows; SPSS Inc., Chicago, IL, USA). Categorical variables were compared in two groups by using Pearson Chi-square test and the results are indicated as frequency and percent. An independent sample t-test was performed to compare normally distributed variables in two groups and these variables are indicated as mean ± standard deviation (SD). If continuous variables were not normally distributed, Man Whitney U test would be used and data would be indicated as median (minimum - maximum). P values less than 0.05 were accepted as statistically significant.

Results
The demographic and laboratory characteristics of the patient and control groups are summarized in Table 1.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>TOTAL (87)</th>
<th>RA (45)</th>
<th>Control (42)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Year)</td>
<td>50.24 ± 12.84</td>
<td>50.67 ± 12.22</td>
<td>49.84 ± 13.42</td>
<td>0.762</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.18 ± 15.66</td>
<td>76.18 ± 15.13</td>
<td>78.11 ± 16.17</td>
<td>0.562</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.8 ± 9.57</td>
<td>162.42 ± 9.78</td>
<td>163.27 ± 9.37</td>
<td>0.677</td>
</tr>
<tr>
<td>WBC</td>
<td>6.94 ± 2.03</td>
<td>7.25 ± 2.41</td>
<td>6.65 ± 1.68</td>
<td>0.175</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>13.15 (9.48-121)</td>
<td>13.1 (9.48-121)</td>
<td>13.2 (10.04-15.96)</td>
<td>0.835</td>
</tr>
<tr>
<td>Sedimentation (mm/hour)</td>
<td>21.47 ± 17.48</td>
<td>27.88 ± 24.00</td>
<td>16.59 ± 11.39</td>
<td>0.006</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>978.76 ± 15.47</td>
<td>98.14 ± 15.41</td>
<td>97.41 ± 15.52</td>
<td>0.827</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>109.24 (42-747)</td>
<td>102 (50-250)</td>
<td>116 (42 - 747)</td>
<td>0.263</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>197.7 ± 38.08</td>
<td>196.4 ± 42.69</td>
<td>199.02 ± 33.78</td>
<td>0.753</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>50.9 ± 14.54</td>
<td>55.67 ± 16.67</td>
<td>46.45 ± 12.56</td>
<td>0.005</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>118.5 (43.4-198.2)</td>
<td>111.6 (43.4-198.2)</td>
<td>125 (65.6-178)</td>
<td>0.182</td>
</tr>
<tr>
<td>CRP (mg/ml)</td>
<td>1.03 ± 2.15</td>
<td>1.7 ± 4.06</td>
<td>0.41 ± 0.38</td>
<td>0.036</td>
</tr>
<tr>
<td>RF (IU/ml)</td>
<td>27.32 ± 28.23</td>
<td>45.78 ± 57.8</td>
<td>10.10 ± 0.63</td>
<td>0.226</td>
</tr>
<tr>
<td>Insulin (µg/mL)</td>
<td>9.35 (2.46-535)</td>
<td>8.81 (2.93-535)</td>
<td>9.85 (2.46-61.78)</td>
<td>0.659</td>
</tr>
<tr>
<td>Omentin (ng/mL)</td>
<td>512.6 (47.8-2181.8)</td>
<td>539.4 (47.8-2181.8)</td>
<td>487.6 (279.4-784.9)</td>
<td>0.259</td>
</tr>
</tbody>
</table>

Table 1: The demographic and laboratory characteristics of the patient and control groups.  

HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, RF: Rheumatoid Factor, CRP: C-Reaktif Protein, WBC: White Blood Cell

The study population consisted of 87 cases with a mean age of 50.24 ± 12.84. Of those 87 cases, 45 cases with a mean age 49.84 ± 13.42 were patient and 42 cases with a mean age 50.67 ± 12.22 belong to the control groups, respectively. There was no statistical significance between mean age, weight, and height features of the two groups (p>0.05). No statistical significance was determined between the groups for some laboratory parameters like White blood cell (WBC), glucose, triglyseride, total cholesterol, Low-density lipoprotein (LDL), and insulin (p>0.05). However, some other laboratory parameters including sedimentation, High-density lipoprotein (HDL), CRP and RF levels in patient group were determined to be significantly higher compared to the control group (p<0.05). There was no significant difference between the patient (539.4 (47.8-2181.8)) and control (487.6 (279.4-784.9)) groups in terms of serum omentin level (p>0.05).

The Omentin Val109Asp genotypes distributions are shown in Table 2. In the patient group, Asp/Asp, Val/Asp, and Val/Val genotypes were found in 25 (55.55%), 19 (42.22%), and 1 (2.23%) of the cases, respectively. In the control group, Asp/Asp and Val/Asp genotypes were found in 22 (52.38%) and 20 (47.62%) of the cases, respectively. The Val/Val genotype was not determined in control group.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Total (87)</th>
<th>Control (42)</th>
<th>RA (45)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp/Asp n (%)</td>
<td>47 (54.02)</td>
<td>22 (52.38)</td>
<td>25 (55.55)</td>
<td></td>
</tr>
<tr>
<td>Val/Asp n (%)</td>
<td>39 (44.83)</td>
<td>20 (47.62)</td>
<td>19 (42.22)</td>
<td>0.456</td>
</tr>
<tr>
<td>Val/Val n (%)</td>
<td>1 (1.15)</td>
<td></td>
<td>1 (2.23)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Omentin Val109Asp SNP genotypes distributions in the patient and control groups.

Val/Val=GTC/GTC, Val/Asp=GTC/GAC, Asp/Asp=GAC/GAC

There was no significant relationship between the control group and the patient group in terms of the genotype frequency.

Discussion and conclusion
Rheumatoid arthritis, a chronic inflammatory disease, is caused by synovial inflammation followed by cartilage destruction and consequent loss of peripheral joint function. Symptoms asso-
associated with RA reduce the quality of the patient’s life[18, 19]. The irreversible destruction of cartilage components is considered to be the critical point of the RA, and a method for prevention of this destruction has not been developed yet[20]. Therefore, the markers for early diagnosis of the RA have been the focus in this study. The biochemical markers have also become the focus in this study because of the clinically useful non-invasive diagnostic tools for monitoring the changes in cartilage turnover in RA patients[21]. It is well established that RA is an autoimmune disease with a complex network of interactions between cognate and innate immunity, and numerous immune cells and mediators of inflammation are present in the synovial tissue and fluid. Chemokines and cytokines are currently considered therapeutic targets for RA because of their involvement in the pathogenesis of RA[22, 23]. Adipocytokines, a class of cytokine, is derived from white adipose tissue (WAT), but it is also synthesized in tissues other than WAT. They have several functions including modulation of immunological and inflammatory pathways. The role of adipocytokines in the pathogenesis of inflammatory diseases like RA has been extensively investigated for the last ten years[24, 25].

The first discovered and most studied adipokine is leptin. In addition its involvement in the regulation of body weight, a catabolic role of it in cartilage metabolism has also been proven by Bao et al.[26]. Even though no significant relationship was reported between serum leptin levels and RA by Wistowska et al.[27], this result was supported by two studies conducted by Targońska-Stepniak et al. In these studies, increased serum leptin level in RA patients was obtained. There was relationship between the risk of aggressive course of RA and increased leptin levels. It can be concluded from these results that leptin shows inflammatory effect, and it is likely to play a role in the pathogenesis of RA[28, 29].

Adiponectin can be a putative biomarker for diseases because of its role in several biological processes from head to toe, and five isoforms of it have been identified up to date. Several investigations have been carried out to evaluate its functions, but its possible involvement in inflammation is still under debate. It has been found to be negatively correlated with several diseases like cardiovascular diseases, diabetes and obesity. In contrast, significantly increased serum level of adiponectin was observed in RA patients with disease duration of less than 10 years[29, 30]. The correlation between elevated serum adiponectin level and RA disease severity was also proven in several studies[31, 32].

Among the adipokines, resistin and visfatin are indicated to play roles in inflammation and auto-immune diseases as important pro-inflammatory mediators, and so they become potential target for RA[33]. Resistin was characterized in 2001, and its effective involvement in RA has been shown[34, 35]. The inflammatory effect of the resistin was also investigated by Zhang et al., who cultured human articular chondrocytes with resistin and measured the expression levels of the matrix metalloproteinases (MMPs). The increased expression levels of MMPs were determined through the up regulation of cytokines and chemokines[36]. Similarly, the elevated expression level in RA and inflammatory property in chondrocytes were established in detail[37, 38]. Therefore, visfatin is also potential therapeutic target for RA disease[39].

Another comprehensive study was performed over 167 patients with RA and 91 control subjects relating to serum adipokines levels by Rho et al., who reported increased levels of leptin, resistin, adiponectin and visfatin[40]. These results were the main motives behind investigating the possible involvement of the omentin in RA in this study. To this end, serum omentin level and Omentin Val109Asp polymorphism in the pathogenesis of RA in Turkish population were investigated. Omentin is the last discovered adipokine, and its potential role in the inflammatory disease is under debate even though there are several studies carried out in order to prove anti-inflammatory effect. It was reported in a recent study that synovial fluid level of omentin was significantly lower in RA than osteoarthritis[41]. So, omentin might also be a putative biomarker for RA. The experimental results in this study demonstrated that there was no significant difference in terms of the serum omentin level and Val109Asp polymorphism. This can be attributed to the patient groups. The patient group consisted of RA patients using medicine, and these medicines may elevate and suppress the serum level of omentin. Another reason might be the control group subjects. Control groups consisted of subjects who applied to the PMR Clinic due to various complaints other than inflammatory reasons, and they were not fully healthy. These circumstances might change the serum omentin level in RA. To elucidate the putative role of the omentin in the pathogenesis of RA, this study should be conducted on a larger
population with more appropriate subjects.

References

12) Yang RZ, Shuldiner AR, Gong DW. Cloning of omentin, a new adipokine from human omental fat tissue. NCBI nucleotide database, 2003; Accession Number: AY549722.


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