Gouty arthritis is an inflammatory disease triggered by the accumulation of monosodium urate crystals in the joints [1]. Gouty arthritis is one of the most common inflammatory arthritis in the world, reported at a rate of 1-2% among men over 30 years old and women over 50 years old, depending on ethnic variations [2]. Acute gouty arthritis generally affects the first metatarsophalangeal joint and shows an intermittent disease course. Chronic tophaceous gouty arthritis reveals itself usually after many years and can cause deformities in the joints [3]. In recent years, the role of the inflammasome complex in the pathogenesis of the disease has been revealed. Gouty arthritis initiates with the deposition of monosodium urate crystals in the joints, activation of the inflammasome complex, and increased expression of proinflammatory cytokines, such as interleukin-1 primarily [4]. Human nucleotide binding and oligomerization domain (NOD) protein family, with almost 25 members, is responsible for apoptosis and signal transduction. NOD2, a member of the NOD family, is a cytoplasmic molecule recognizing muramyl dipeptide, a peptidoglycan contained in the wall structure of Gram-positive and Gram-negative bacteria, and controlling apoptosis and inflammatory processes [5]. NOD2 molecule plays an important role in the etiopathogenesis of many inflammatory diseases (e.g., Crohn’s disease [CD]), and induces predisposition to the disease [6].

Among these diseases, two rare non-caseating granulomatosis, Blau syndrome and early-onset sarcoidosis, caused by sequence variants in the caspase recruitment domain-containing protein 15 (NOD2/CARD15) gene, have been included in the group of nuclear factor-κB (NF-κB) activation disorders or autoinflammatory granulomatous diseases [7]. Further, studies focusing on the genetic background in CD have highlighted its susceptibility in patients carrying several
CARD15/NOD2 polymorphisms [8]. Moreover, a positive association with other CARD15/NOD2 gene polymorphisms has been recently described in a systemic syndrome, termed NOD2-associated autoinflammatory disease [9]. NOD2 is often expressed in epithelial cells such as Panetta cells in the small intestine, as well as in myeloid cells such as macrophages, neutrophils, and dendritic cells. The expression of NOD2 is induced by some of proinflammatory cytokines (e.g., tumor necrosis factor-alpha and interferon-gamma) [10], however, NOD2 per se activates NF-κB and induces the genes encoding proinflammatory cytokines [11].

NOD2/CARD15 gene polymorphisms are important for inflammatory bowel diseases; however, they have not been evaluated in rheumatologic diseases in detail. This study aimed to investigate NOD2/CARD15 gene mutations in patients with gouty arthritis and to determine any possible correlation between the NOD2/CARD15 gene mutations and susceptibility to the disease, severity, and symptoms of the disease.

MATERIALS AND METHODS

Ninety-three patients with gouty arthritis (72 males and 21 females) and 51 healthy volunteers (38 males and 21 females) matched for age, gender, and ethnicity were included in the study. The patients were diagnosed according to the criteria of American College of Rheumatology and followed up in the Orthopedics and Rheumatology clinic of Sifa University. All patients and healthy volunteers were Turkish in origin, living in the Aegean Region of Turkey. The study was supported by the Scientific Research Projects Unit of Sifa University, approval by the Ethics Committee and consent forms from all patients and volunteers were obtained. A detailed medical history was taken from all patients, and systemic examination as well as the examination of locomotor system was performed. Demographic data (age, gender), clinical characteristics (disease duration, frequency of attacks, arthritis pattern, and comorbid disease), and laboratory findings (serum uric acid, erythrocyte sedimentation, C-reactive protein) were recorded (Table 1).

**Molecular analysis**

Samples (2 mL) of whole blood were collected into ethylenediaminetetraacetic acid (EDTA)-anticoagulated tubes using the standard venipuncture method. Genomic DNA was extracted from the EDTA-anticoagulated whole blood samples using the QIAmp Blood DNA Mini kit (Qiagen, Hilden, Germany), following the manufacturer’s instructions. The NOD2/CARD15 R702W and G908R gene mutations were explored by the polymerase chain reaction restriction fragment length polymorphism method while the 3020insC mutation was analyzed by DNA sequencing [8].

**Statistical analysis**

Data were analyzed by the Statistical Package for the Social Sciences (SPSS) 20.0 software for Windows (SPSS, Chicago, Illinois, USA). Cross tables were used in the analysis of data and Chi-square and Fisher’s exact test analyses were performed where appropriate. The data are given as frequencies and percentages. A statistical significance threshold was considered as p < 0.05.

**RESULTS**

The mean age of the patients was 54.2 ± 14.2 (21-78) years and mean duration of the disease was 3.1 ± 2.9 years. Clinical and laboratory evaluation of the patients with gouty arthritis revealed first metatarsophalangeal joint involvement in 72 (77.4%), ankle arthritis in 43 (46.2%), knee arthritis in 20 (21.5%), finger joint involvement in 18 (19.5%), elevated levels of serum uric acid in 76 (81.7%), elevated erythrocyte sedimentation rate in 45 (48.4%), and increased serum C-reactive protein in 42 (45.2%) patients (Table 1). Comorbid diseases, i.e., diabetes mellitus and hypertension were present in 10 (10.7%) and 18 (19.5%) patients, respectively. Four (9%) heterozygous mutations were detected in the NOD2/CARD15 G908R and NOD2/CARD15 R702W genes, while the NOD2/CARD15 C3020ins displayed no mutations (Table 2). When compared with the control group, any statistically significant difference was not detected for each of the three DNA regions. There was no statistically significant correlation between the NOD2/CARD15 mutations and clinical signs or laboratory parameters in the patient group (p > 0.05).

**TABLE 1. Clinical and laboratory characteristics of patients with gouty arthritis (n=93)**

<table>
<thead>
<tr>
<th>Clinical and laboratory characteristics</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st metatarsophalangeal joint arthritis</td>
<td>72 (77.4)</td>
</tr>
<tr>
<td>Finger joint arthritis</td>
<td>18 (19.4)</td>
</tr>
<tr>
<td>Ankle joint arthritis</td>
<td>43 (46.2)</td>
</tr>
<tr>
<td>Knee arthritis</td>
<td>20 (21.5)</td>
</tr>
<tr>
<td>Elevated serum uric acid level</td>
<td>76 (81.7)</td>
</tr>
<tr>
<td>Elevated serum erythrocyte sedimentation rate</td>
<td>45 (48.4)</td>
</tr>
<tr>
<td>Elevated serum C-reactive protein level</td>
<td>42 (45.2)</td>
</tr>
</tbody>
</table>

**TABLE 2. NOD2/CARD15 gene mutations in patients with gouty arthritis (n=93) and controls (n=51)**

<table>
<thead>
<tr>
<th>Mutations</th>
<th>n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals without any mutations</td>
<td>89 (95.7)</td>
<td>0.354</td>
</tr>
<tr>
<td>Homozygote individuals</td>
<td>0 (0.0)</td>
<td>0.350</td>
</tr>
<tr>
<td>Heterozygote individuals</td>
<td>4 (4.3)</td>
<td>0.564</td>
</tr>
<tr>
<td>Individuals with R702W allele</td>
<td>2 (2.1)</td>
<td>0.583</td>
</tr>
<tr>
<td>Individuals with G908R allele</td>
<td>2 (2.1)</td>
<td>0.452</td>
</tr>
<tr>
<td>Individuals with 3020insC allele</td>
<td>0 (0.0)</td>
<td>0.350</td>
</tr>
</tbody>
</table>
DISCUSSION

This study has revealed that the NOD2/CARD15 gene mutation frequency is low among the Turkish patients with gouty arthritis. In total, four heterozygous mutations (4.3%) were detected in all the three DNA regions, while homozygous mutation was not detected in any DNA region. No correlation was found between the NOD2/CARD15 mutations and clinical findings. A possible role of the NOD2/CARD15 gene mutations in patients with gouty arthritis has not been studied before, and in fact, our study features the first study in the literature on this topic. Different studies have tried to demonstrate that the NOD2/CARD15 gene mutations might play a role in the pathogenesis of many chronic inflammatory diseases, but conflicting results have been obtained. Kim et al. found the frequency of a NOD2/CARD15 mutation as 0.5% in 205 Korean ankylosing spondylitis patients and suggested that it is not a risk factor for the development of ankylosing spondylitis [12]. van der Paardt et al. studied CARD15 gene mutations in patients with ankylosing spondylitis and found no statistically significant difference when compared with the control group [13]. Laukens et al. studied a NOD2/CARD15 gene mutation in 104 patients with spondyloarthropathy and found that mutation frequency was similar to the control group [14]. However, the comparison of the gene mutation in spondyloarthropathy subgroup, having intestinal inflammation confirmed with colonoscopy, with the control group, revealed a statistically significant difference. Hence, the presence of the NOD2/CARD15 mutation was considered to be a probable risk factor for the development of chronic bowel inflammation. Furthermore, a relation between a NOD2/CARD15 gene mutation and Crohn-related sacroiliitis was also identified, emphasizing the correlation between intestinal and articular inflammation [15]. Crane et al. studied a NOD2/CARD15 gene mutation in patients with ankylosing spondylitis, ulcerative colitis, and CD, and could not find any statistically significant differences between all three disease groups [16]. In addition, a NOD2/CARD15 gene mutation was studied in 100 Turkish patients with ankylosing spondylitis [17] and in 243 Polish patients with rheumatoid arthritis [18]; none of these studies showed a statistically significant difference when compared to the healthy population.

CONCLUSION

The results of this study suggest that the NOD2/CARD15 gene mutations probably do not contribute to the development of gouty arthritis. However, there are some major limitations of our study. First of all, the patients were not examined during the attacks when the inflammation exacerbated. Second, the relationship with different disease phenotypes was not considered. Finally, the study has been conducted with few patients and healthy controls and therefore, power of the study is considerably low with this small sample set. Furthermore, the study included a limited analysis of the NOD2/CARD15 gene, with respect to disease-associated alleles. Additional multicenter prospective studies are required, with larger series of patients to empower the findings of the study.

ACKNOWLEDGMENTS

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DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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Ahmet Karaarslan, et al.: NOD2/CARD15 gene mutations in gouty arthritis

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