Investigation of Paraoxanase Enzyme Polymorphism in Patients with Alopecia Areata

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ABSTRACT

Objective: In this study, we investigated the relationship between AA and genetic polymorphisms of PON1 R192Q and PON1 L55M.

Methods: One hundred and nineteen patients with AA and 104 healthy controls were included in this study. The changes in aminoacid squencies on codon 55 and 192 were analysed by the polymerase chain reaction and restriction enzymes.

Results: The frequencies of M, L homozygotes and ML heterozygotes on PON 55 region were 11.8%, 58% and 30.2%, respectively in the patient group while they were 12.5%, 43.3% and 44.2%, respectively in the control group. The frequencies of R, Q homozygotes and RQ heterozygotes on PON1 192 region were 10.9%, 41.2% and 47.9%, respectively in the patient group while they were 9.6%, 42.3% and 48.1%, respectively in the control group. While the frequency of PON 55 L allele was higher in patient group than control group, there were no differences in the frequency of 192 R allel between the groups ($p = 0.07$, $p = 0.947$, respectively).

Conclusions: There were no statistically significant relations between the AA and the PON1 ML55 and QR192 polymorphism. Although the frequency of PON 55 L allel was higher in the patient group than control group, there were no relation between the AA and both PON1 M/L55 and R/Q192 polymorphism.

Keywords: Alopecia areata, paraoxonase-1, polymorphism

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INTRODUCTION

Alopecia areata is a common, recurrent, chronic inflammatory disorder of the hair follicles and sometimes the nails. Alopecia areata characterized by nonscarring lesions and partial loss of scalp hair. The prevalence and lifetime risk of AA is approximately 0.2% and 2%, respectively (1). The pathogenesis of AA is still unknown. It is thought that genetic, psychological and autoimmune factors, oxidative stress and neuropeptides may be responsible for pathogenesis of AA (2).

Paraoxonase (PON) is an enzyme with glycoprotein structure that depends on calcium. The PON gene family contains three different members (PON1, PON2 and PON3). Paraoxonase-1 is synthesized by the liver and secreted into blood. Paraoxonase-1 enzymes, locate on high density lipoproteins (HDL) in the serum and prevent the oxidation of LDL.

The activity of PON1 is affected by genetic and environmental factors (3). Mainly two polymorphisms are defined in the PON1 gene. One of them is the (A/G) polymorphism of PON and results in glutamine (Q) to arginine (R) substitution at codon 192. PON1Q genotype has lower PON activity, whereas 192 R has a higher hydrolytic activity toward paraoxon. The other polymorphism takes place at codon 55 and results in Leu(L) to Met(M) substitution and has been associated with plasma PON1 protein levels. In recent studies, the relation between PON polymorphism and oxidative stres related disorders like cardiovascular disease, diabetes mellitus, some cancers and psychiatric disorders has been investigated.

In this study, we examined the relationship between AA and genetic polymorphisms of PON1R192Q and PON1L55M. To our knowledge, this is the first study examined PON1 polymorphisms in patients with AA.

MATERIALS AND METHODS

Subjects

The study group consisted of 119 patients with AA (68 males and 51 females; mean age: 32.5 ± 9.3 standard deviation [SD] years), and 104 (55 males and 49 females; mean age: 32.1 ± 10.2 SD years) healthy controls with no scalp lesions in their personal history or on clinical examination. Participants fort this study were drawn from patients seeking treatment at the Dermatology outpatient clinic of Gaziosmanpasa University, Tokat, Turkey. All participants were questioned and examined for clinical data including demographics, alcohol use, smoking, family history, age of onset, presence of any autoimmune, psychiatric and infectious diseases. Associated nail findings, numbers of the episodes experienced, localization (scalp, beard/mustache, eyebrow, eyelash) and the severity of AA also noticed. Patients having diagnostic criteria for AA were included in this study. Alopecia areata clinical subtype was determined according to the AA investigational assessment guidelines. Patients using topical, intralesional, or systemic agents as steroids or immunosuppressives are likely to cause regrowth in AA within the past month, patients subjected to sessions of PUVA for at least six months before this study were excluded.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The protocol was approved by the Ethics Committee of Namik Kemal University Faculty of Medicine, and informed consent was obtained from all all individual participants included in the study.

Genotyping

Genomic DNA was extracted from peripheral leukocytes from EDTA-anticoagulated blood using the High Pure Polymerase Chain Reaction Template Preparation Kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. To identify PON1 L55M and Q192R single-nucleotide polymorphisms (SNPs), genotyping was performed using commercially synthesized primers and fluorescently labeled probes (Metabion, Martinsried, Germany) and the LightCycler 480 II Real-Time Polymerase Chain Reaction System (Roche Diagnostics). The genotyping method was based on methods developed previously (4) for genotyping both PON1 55 and 192 polymorphisms using LightCycler real-time polymerase chain reaction technology, which relies on fluorescence resonance energy transfer. Target fragments of the human PON1 gene were amplified with specific primers. Melting curves were transformed to melting peaks by plotting the negative derivative of the fluorescence signal *versus* the temperature. The genotypes were identified by creating a melting curve with specific melting points.

Statistical analysis

Either the $\chi 2$ test or Fischer's exact test was used to compare the distribution of the *PON1* polymorphisms of patients with RVO with those of the healthy controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated whenever the Chi-squared or Fischer's exact test was significant. Significant probability values were also corrected for multiple testing (Bonferroni correction; Pc). The $\chi 2$ test was used to evaluate Hardy-Weinberg equilibrium for the distribution of the genotypes of the patients and the controls. Categorical data were expressed as counts and percentages. According to the Kolmogorov-Smirnov normality test, the two independent sample Student *t*-test was used to compare continuous data between two groups. Continuous data are expressed as mean ± standard deviation (SD).

P-values below 0.05 were considered statistically significant. Statistical analysis was performed using commercial software (PEPI 3.0; IBM SPSS Statistics 19, SPSS Inc., Somers, NY).

RESULTS

The study population consisted of 119 patients with AA who presented to the dermatology clinic at Gaziosmanpasa University Hospital and 104 age-matched controls. The mean age was 64.6 ± 7.7 (age range; 50–88) years for the control group and 63.6 ± 8.2 (age range: 32–82) years for the patient group (p = 0.347). Age and gender were not different between patient and control groups. The distribution of the genotype and allele frequencies of *PON1* L55M and *PON1* Q192R in patients with AA and the controls is shown in Table 1.

Table 1: The frequencies of the genotypes and allels of the groups

Genotype/allele	**AA (n = 119)**		**Control (n = 104)**	
	n	%	n	%
PON1 55 L/M Genotypes				
LL	69	58.0	45	43.3
LM	36	30.2	46	44.2
MM	14	11.8	13	12.5
Allele frequency				
L	174	73.1	136	65.4
M	64	26.9	72	34.6
PON1 192 Q/R Genotypes				
QQ	49	41.2	44	42.3
QR	57	47.9	50	48.1
RR	13	10.9	10	9.6
Allele frequency				
Q	155	65.1	138	66.3
R	83	34.9	70	33.7

AA: Alopecia areata

As shown in Table 1, QQ, QR and RR genotype frequencies in the controls were 42.3%, 48.1% and 9.6%, respectively while those of AA group were 41.2%, 47.9% and 10.9%, respectively. The frequencies of MM homozygote, LL homozygote and LM heterozygote in the AA group were 11.8%, 58% and 30.2%, respectively while those of control group were 12.5%, 43.3% and 44.2%, respectively. The LL genotype occurred more frequently in patients with AA than in controls (58% versus 43.3%) but the difference was no statistically significantly (p = 0.07; Table 1). These results demonstrated that the distribution of genotype frequencies for PON1Q192R and PON1L55M were similar for both groups (Table 1).

Accordingly, there was no significant difference in both L/M and Q/R genotype distributions between AA and control groups. However, Q and L were the most frequent alleles in both groups. The least frequent allels in AA and control grops were M (26.9%) and R (33.7), respectively.

DISCUSSION

Reactive oxygen species (ROS) are quite harmful molecules and and play crucial role in the oxidative damages of tissues or cells. The cells have antioxidant defence systems that neutralize ROS. The systems may be nonenzymatic such as vitamin E, glutathion and selenium, or enzymatic such as superoxidase dismutase, catalase and paraoxanase. Oxidant and antioxidant systems are in equlibrium in the cell. If this balance is disrupted in favour of oxidant compounds, oxidative cell damages occur.

It is known that oxidative damage plays an mportant role in many dermatological disorders. The effects of oxidative stres in AA has been investigated by many other researchers. It is reported that serum malondialdehyde levels in patients with AA are higher than control groups in previous studies (5, 6). Corresponding to them, some researchers declared a decrease in activities of antioxidant enzymes such as catalase, superoxide

dismutase and PON in AA patient groups compared with controls (7, 8). The reasons for the decrease in antioxidant enzymes activities have not been clarified. This conditions may be due to inadequately synthesized proteins and polymorphisms.

Until now, many studies have been carried out to investigate the relation between antioxidant enzyme activities, lipid peroxidation and the pathogenesis of AA. However, there is not any study about the polymorphisms of antioxidant enzymes in AA patients. Paraoxonase-1 is secreted by the liver and carried on HDL in blood. The enzyme is responsible for antioxidant effect of HDL. It is demonstrated that PON inhibited the oxidation of in vitro studies (8).

In the literature, there are only two studies about the relation of PON activity and AA. These two studies showed that patients with AA have lower activity of PON compared to healthy controls (9, 10). The reason for this decreasing activity of PON is obscure. This decreased activity of PON may be due to (i) the presence of some factors that inhibit the enzyme activity, (ii) inadequate production of PON, (iii) abnormal structure and expression of the enzyme. We hope that this study will contribute to make some progress on the most challenging aspect of pathogenesis of AA .

The PON1 gene is located on the long arm of chromosome seven at band 21–22. The most common polymorphisms of the PON1 gene are the glutamine/arginine substitution (Q/R) at codon 192 and the leucine/methionine substitution (L/M) at codon 55. The 55 L/M polymorphism is associated with the synthesis of PON rather than interaction of PON1 with its substrates. Paraoxonase-1 55 L is the most common allele in the population and plays a key role in correct packaging of protein (11). In this study, the PON1 55 L/M genotypes and L allel frequencies of AA patients and control subjects were similar. These results show us that there is not any difference between patient group and control group according to protein

contration. Hence, the reduction in the enzyme activity which is reported by the previous studies may not be due to decrease in enzyme synthesis.

The studies that establish no differences between patients with AA and healthy controls according to genotype and activity of PON support our hypothesis (12, 13). In these studies there were not any differences between control groups and patient groups according to frequencies of genotypes which act on protein synthesis and enzyme activities. Although there were no statistically significant differences in the allel frequencies there were suppresions in the activity of enzymes between two groups. This suppression may be associated with increased amount of inhibitory substances in the microenvironment, such as reactive oxygen species.

In this study, the frequencies of PON1 192 QQ and QR genotypes were similar in the AA patients and control groups. The most common haplotypes in the control group and AA group were QR/LM and QR/LL, respectively. In AA patients Q and R allele frequencies were not significantly different from those of the controls. The RR genotype was the least frequent genotype in both groups. In studies of Turkish population showed that, there were differences between the control and patient group with respect to frequencies of QR (12, 14, 15) and QQ genotypes (16–18) in spite of the least frequent genotype was RR in all groups.

The prevalance of PON1 192R allele was 0.34 in control group compared with 0.35 in AA group. The homozygosity rate of PON1 192RR allele may show differences between the regions of a country (19–23). In the literature, there is no study related with PON1 polymorphism in AA patients. Therefore, to the best of our knowledge, this is the first study about the PON1 polymorphism in AA patients. Asefi *et al* (24) asserted that PON1 55 M allele is a risk factor for the psoriasis. Dursun *et al* (25) reported that there was a relation between PON1 55 M allele polymorphism and Behçet disease. Although there were no significant differences between the groups with respect to PON1 L55M polymorphism,

presence of increased frequency in AA group makes us think, there may be a weak relation between PON1 L 55 M polymorphism and AA.

There are some limitations to this research study such as (i) the enzyme activity was not to be measured, (ii) it was a very limited number of cases, (iii) cases with RR and MM allele was also very limited. Additional studies, inclusive of a larger sample size, are needed to extend these initial findings. Although these limitations, we think that every study that examine polymorphic structures showing great variations within and between populations are very precious and contribute to knowledge in society.

In conclusion, although the frequency of PON 55 L allele was slightly higher in AA patients, there was no relation between AA and PON1 ML55 and RQ192 polymorphisms.

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