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Investigation of TNF-alpha gene (G308A) and GSTP1 gene (Ile105Val) polymorphisms in Turkish patients with retinopathy of prematurity

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Background/aim: Retinopathy of prematurity (ROP) is one of the most frequent causes of blindness in newborn babies. Currently, its etiology is not fully understood. In this study we aimed to investigate the correlation between a patient group with ROP and a control group in terms of the tumor necrosis factor-alpha (TNF-alpha) (G308A) gene and glutathione-S-transferase P1 (GSTP1) (Ile105Val) gene polymorphism.

Materials and methods: Sixty-two patients diagnosed with ROP and 58 control subjects were included in this study. For TNF-alpha (G308A) gene and GSTP1 (Ile105Val) gene polymorphisms, the polymerase chain reaction-restriction fragment length polymorphism method was used. In statistical analysis the significance level was determined as $P < 0.05$.

Results: When the patient and control groups were compared in terms of TNF-alpha (G308A) gene and GSTP1 (Ile105Val) gene polymorphisms, no statistically significant difference was found ($P > 0.05$).

Conclusion: In our study, no correlation was identified between TNF-alpha (G308A) gene and GSTP1 (Ile105Val) gene polymorphisms and susceptibility for development of ROP. Further studies are required with more cases of ROP patients and other gene polymorphisms that could be related.

Key words: TNF-alpha, GSTP1, gene polymorphism, retinopathy of prematurity

1. Introduction

Retinopathy of prematurity (ROP) is one of the main causes of blindness in childhood and its pathogenesis is not fully understood. It a disease that develops in relation to abnormal proliferation of retinal capillaries, which are observed in newborn babies (1,2). Currently its etiology is not fully understood. It is thought that factors like the existence of retinal capillaries that have not fully completed their development, low birth weight, age of pregnancy, hyperoxia caused by oxygen treatment, patent ductus arteriosus (PDA), bronchopulmonary dysplasia, apnea that is repeated and requires mechanical ventilation, hypercapnia and hypocapnia, sepsis, and genetic susceptibility play a role in development of this condition (3–5).

There is a strong correlation between ROP and low birth weight and short gestational age. In addition to well-established environmental factors, such as early oxygen exposure and gestational age, there is also a strong genetic susceptibility to ROP. Clinical development of ROP may vary with infants. While some of patients with ROP may develop advanced stages of ROP progression, some may have spontaneous regression. In some studies it was demonstrated that these variations may be caused by genetic factors (6–9).

Glutathione-S-transferase (GST) enzymes, which are encoded by GST genes, are responsible for the detoxification of chemicals found in the environment and naturally synthesized metabolites, and they play an important role in protecting tissue from oxidative damage. There are studies regarding the role of stress on diabetic retinopathy development. The retina, a tissue rich in polyunsaturated fatty acid, uses more oxygen than any other tissue in the body and is very susceptible to damage (10–13). In our previous study, we investigated the GSTT1 and GSTM1 null polymorphisms, which play

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a role in the antioxidant defense system of patients with ROP, and could not identify any correlation (14). In this study, we have investigated another polymorphism related to Ile105Val located on the GSTP1 gene that might have an impact on GST enzyme activity, because there are studies that investigate the correlation between GSTP1 Ile105Val polymorphism and cancer and various other diseases. Qadri et al. observed a significant correlation between the Val/Val GSTP1 variant and predisposition to prostate cancer in the Kashmiri population (15). In the study by Çalıkoglu et al. regarding the GSTP1 variant Val/Val genotype, they demonstrated that the development risk of chronic obstructive pulmonary disease increased 4 times when compared with the control group (16). In the study conducted by Inada et al., correlation was identified between GSTP1 polymorphism and early onset nephropathy (7).

Tumor necrosis factor-alpha (TNF-alpha) is one of the major inflammatory and immune regulators. TNF-alpha is mainly secreted by monocytes/macrophages. In previous studies, a relation was demonstrated with the adenine-guanine polymorphism on codon 308 of the TNF-alpha promoter region (17–19). In the literature it has been demonstrated that the TNF-alpha (G308A) polymorphism has a tendency for ulcerative colitis, Crohn's disease, and certain infectious diseases (20–22). In a recent study, it was indicated that G238A, which is another polymorphism of the TNF-alpha gene, is a potential factor in the pathogenesis of diabetic retinopathy (23).

In this study, we aimed to investigate the correlation between a patient group with ROP and a control group in terms of the TNF-alpha (G308A) gene and GSTP1 (Ile105Val) gene polymorphism.

2. Materials and methods

2.1. Patients

Our study consisted of 62 patients with ROP and a control group of 58 prematurely born infants who did not show ROP. Our study group included either premature babies with a birth weight of 1500 g or lower born in week 32 or earlier, or babies with a birth weight of between 1500 and 2000 g or who were born after the 32nd week with unstable clinical status, requiring support for respiration/circulation and declared as being at high risk by the neonatologist/pediatric specialist monitoring the babies. Patients that met the same criteria but were not diagnosed with retinopathy and had no eye problems were included in the control group.

The patients were put through fundus examinations using indirect ophthalmoscopy. Before this examination, 2.5% phenylephrine and 0.5% tropicamide drops were applied twice at 15-min intervals for pupil dilatation. The first scanning examination was conducted on all the babies when their chronological age was 4–6 weeks. Patients in whom premature retinopathy was not detected were reevaluated at 2-week intervals until the retinal vascularization was complete. Where ROP was diagnosed, the babies were examined every week starting from the beginning of the disease until it regressed. For threshold ROP disease treatment, laser photocoagulation under general anesthesia was performed. ROP stages were determined according to the International Premature Retinopathy Classification Committee. We divided the ROP patient group into 2 groups as those in advanced stages (stages 4 and 5) and those in early stages (stages 1, 2, and 3). A hemogram evaluation was simultaneously conducted for all cases in which blood samples were taken for genetic tests and anemic statuses were investigated. This study was approved by the Ethics Committee of the Medical School of Uludağ University.

2.2. Methods

Blood samples from both the patient and control groups were taken in EDTA tubes. Genomic DNA was extracted from whole blood using a DNA isolation kit (Dr. Zeydanlı Life Sciences, Ltd., Turkey) according to the manufacturer's instructions, and samples were stored at –20 °C until polymerase chain reaction (PCR) was performed.

The GSTP1 (Ile105Val) gene polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. For the GST-P1 polymorphism, forward 5'-ACCCCAGGGCTCTATGGGAA-3' and reverse 5'-TGAGGGCACAAGAAGCCCCT-3' primers were used (24). To identify the GSTP1 (Ile105Val) gene polymorphism among the products, the Alw26 I (Genemark, Taiwan) enzyme was used. After digesting the enzyme in 4% agarose gel, the 3 possible genotypes of the GSTP1 allele were determined as follows: if the 176-bp PCR product from the GSTP1 gene was cut into 2 distinct products of 85 bp and 91 bp, then the GSTP1 allele genotype was identified as Val/Val; if the 176-bp PCR product resulted in 3 distinct products of 176 bp, 91 bp, and 85 bp, then the genotype was identified as Ile/Val; and if the 176-bp PCR product was the only product from the PCR procedure, then the genotype was identified as Ile/Ile.

The genotypic analysis of the TNF-alpha G308A polymorphism was performed using a modified version of a previously described PCR-RFLP assay (25). Briefly, G308A genotyping was conducted using the forward primer 5'-AGGCAATAGGTTTTGAGGGCCAT-3' and the reverse primer 5'-TCCTCCCTGCTCCGATTCCG-3', which enabled the use of the restriction enzyme NcoI. PCR products were digested with restriction enzyme NcoI at 37 °C for 16 h and analyzed on 4% agarose gel. When G was present at codon 308, the 107-bp PCR product was cleaved into 2 fragments of 87 bp and 20 bp.

2.3. Statistical analysis

The Shapiro–Wilk test was used to test the normality of variables. An independent samples t-test was used for comparing 2 groups for continuous variables meeting normality assumption, and data were presented as mean ± standard deviation. Otherwise, the Mann–Whitney U test was used and data were presented as median (minimum–maximum) values. Categorical variables were expressed by counts and percentages. Comparisons between the groups were performed with the Pearson chi-square or Fisher exact chi-square test for categorical variables. The significance level was taken as P = 0.05. Statistical analyses were performed with IBM SPSS 20.0 (IBM Corp., USA).

3. Results

In this study, among the 62 cases in the patient group (32 males and 30 females), the birth weight was 1325 ± 326 g, and among the 58 cases in the control group (22 males and 36 females), the birth weight was 1508 ± 298 g, on average (Table 1).

The distribution of genotypes of the G308A polymorphism in the TNF-alpha gene was not significantly different between the ROP patients and the control group (P-value for G/G-G/A = 0.978, G/G-A/A = 0.227, and G/A-A/A = 0.482). No significant differences in genotype frequencies for GSTP1 (Ile105Val) polymorphism were observed between ROP patients and control patients (P-value for Ile/Ile-Ile/Val = 0.942, Ile/Ile-Val/Val = 0.490, and Ile/Val-Val/Val = 0.467) (Table 2).

Table 3 shows the distribution of GSTP1 and TNF-alpha polymorphisms among the early stages and severe stages of ROP patients. No significant differences in genotype frequencies for any polymorphism were observed between ROP patients and control patients, although the Ile/Ile genotype was less frequent in ROP patients.

For the TNF-alpha (G308A) polymorphism, the frequency of the A allele was 9% in the ROP patient group and 13% in the control group. For the GSTP1 (Ile105Val) polymorphism, the frequency of the T allele was 26% in the ROP patient group and 23% in the control group.

Table 1. Clinical characteristics of the ROP patient group and control group.

	ROP patient group n = 62	Control group n = 58
Gestational age at birth (weeks)*	30 (25–33)	32 (27–33)
Birth weight (g)**	1325.58 ± 326.21	1508.79 ± 298.04
Sex***		
Female	30 (48.39)	36 (62.07)
Male	32 (51.61)	22 (37.93)
Hemoglobin levels, g/dL*	10 (7.40–13.50)	10.05 (7.10–14.20)

*: Median (minimum-maximum), **: mean ± standard deviation, ***: n (%).

Table 2. Distributions of the TNF-alpha (G308A) and GSTP1 (Ile105Val) genotypes in the ROP patient group and control group.

Genotypes	ROP patient group n = 62	Control group n = 58	P-value
TNF-alpha (G3008A)			
G/G	51	45	G/G-G/A = 0.978 G/G-A/A = 0.227 G/A-A/A = 0.482
G/A	11	11	
A/A	0	2	
GSTP1 (Ile105Val)			
Ile/Ile (A/A)	36	34	A/A-A/G = 0.942 A/A-G/G = 0.490 A/G-G/G = 0.467
Ile/Val (A/G)	20	21	
Val/Val (G/G)	6	3	

Table 3. Distributions of the TNF-alpha (G308A) and GSTP1 (Ile105Val) genotypes in the early-stage and advanced-stage ROP patients.

Genotypes	Advanced-stage ROP patient group n = 23	Early-stage ROP patient group n = 39	P-value
TNF-alpha (G3008A)			
G/G	20	31	
G/A	3	8	G/G-G/A = 0.516
A/A	0	0	
GSTP1 (Ile105Val)			
Ile/Ile (A/A)	10	26	A/A-A/G = 0.170
Ile/Val (A/G)	10	10	A/A-G/G = 0.353
Val/Val (G/G)	3	3	A/G-G/G = 1.000

The allelic frequencies of both polymorphisms were thus similar in both groups (Figures 1A and 1B).

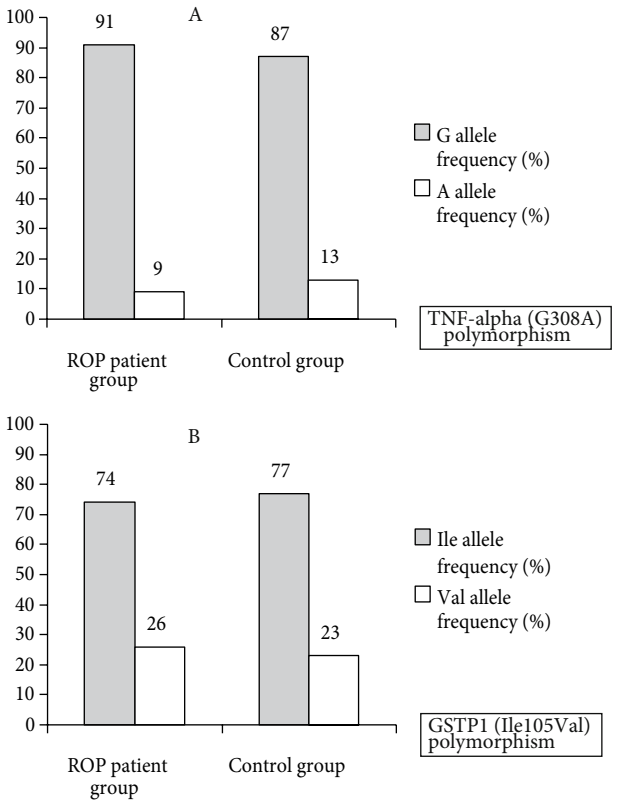

Figure 1. Distribution of the allele frequencies of TNF-alpha (G308A) and GSTP1 (Ile105Val) polymorphisms in the ROP patients and control group. **A)** TNF-alpha (G308A) polymorphism. The gray bars represent the frequency of the C allele and the white bars represent the frequency of the T allele in both groups. **B)** GSTP1 (Ile105Val). The gray bars represent the frequency of the Ile allele and the white bars represent the frequency of the Val allele in both groups.

4. Discussion

ROP is a proliferative vascular disorder of the retina that can lead to visual impairment or complete vision loss in premature infants. Some studies have demonstrated that genetic factors may have major contributions to the etiology and clinical variations of ROP (8,9,26). Here we report the first prospective patient-based study of Turkish ROP patients. In our study, we investigated whether GSTP1 and TNF-alpha gene polymorphisms have an impact on ROP development risk. We showed that there was no correlation in terms of polymorphism when the patient group was compared with the control group.

There are a considerable number of genetic studies that aimed to clarify the pathogenesis of ROP. While in their study Shastry et al. identified a correlation between Norrie disease gene missense mutation and advanced stages of ROP, in the study conducted by Haider et al. such a correlation was not identified in the onset and advanced stages of ROP (27–29). In our previous study of ROP, we identified that GST (GSTM1, GSTT1) and ACE (I/D) gene polymorphisms had no effect on ROP development (14). A study conducted by Mohamed et al. demonstrated that there was a correlation between EPAS1, IHH, AGTR1, TBX5, CETP, and GP1BA genes and ROP (6). While in their study Rusai et al. identified a correlation between the eNOS 27-bp repeat polymorphism and advanced stages of ROP, such a correlation was not identified with the eNOS gene T-786C polymorphism (2). Kwinta et al., Bányász et al., and Shastry et al. could not identify any correlation between ROP development and VEGF polymorphism, while Balogh et al. and Shastry et al. did not identify any significant correlation between IGF-IR gene G(+3174)A polymorphism and ROP (30–34).

In this study, the frequencies of the A allele of -308 in the patient and control groups were determined to

be similar (9% and 13%, respectively; Figures 1A and 1B). This polymorphic variant was shown to affect the promoter region of TNF-alpha, leading to a higher rate of gene transcription than was observed for the -308G allele. Polymorphism in the regulatory region of a gene may lead to variations in gene expression. In our study, there was no association between TNF-alpha (G308A) gene polymorphism and ROP risk (Table 2).

GST is one of the most important enzymes in the enzymatic antioxidant system. GSTP1 gene polymorphism enzyme activity may be different. While there are considerable numbers of studies that investigated the correlation between GST gene variants and diseases like cancer, type 2 diabetes mellitus, coronary artery disease, and chronic obstructive lung disease, as far as we know, to date, there has been no study investigating ROP progress with GSTP1 polymorphism. In the study that we have conducted, when the patient and control groups were compared, no significant difference was identified between GSTP1 Ile/Ile, Ile/Val, and Val/Val genotypes ($P > 0.05$; Table 2).

In our study we divided the ROP patient group into 2 groups, those in advanced stages and those in early stages. While Ile/Ile genotype incidence was low in the advanced-stage ROP group, no correlation was identified in terms of distribution of Ile/Ile and other genotypes ($P > 0.05$; Table 3).

To conclude, as far as we know, this study investigating the relation between ROP and TNF-alpha (G308A) gene and GSTP1 (Ile105Val) gene polymorphisms is the first study of its kind. This study, together with any other similar study that might be conducted in this area, will be a guide in the diagnosis of, treatment for, and protection against this condition. While no significant correlation was identified between ROP and TNF-alpha (G308A) gene and GSTP1 (Ile105Val) gene polymorphisms, the number of our cases was very limited when compared with other polymorphism studies. Thus, the conclusion reached in this study will need to be supported with other studies conducted with higher numbers of cases. Studies conducted with larger series are required to determine if there is a correlation between ROP and the single nucleotide polymorphisms of TNF-alpha and GSTP1 genes.

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