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Effect of Fas -670 A/G Gene Polymorphism on Corneal Allograft Endothelial Rejection

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ABSTRACT

Background: Human cornea expresses functional Fas-ligand capable of killing Fas+ activated lymphocytes. Fas expression is partly regulated by -670 A/G polymorphism in the promoter region of Fas gene. **Objective:** The aim of the present study is to determine the association between Fas-670A/G polymorphism and survival of corneal transplantation. **Methods:** In 276 graft recipients who mainly underwent penetrating keratoplasty because of keratoconus, bullous keratopathy and corneal opacity, Fas -670 A/G polymorphism was determined by allele specific oligonucleotide polymerase chain reaction (ASO-PCR) techniques. **Results:** There was no statistically significant relationship between Fas -670 A/G polymorphism and rejection episode (p=0.35). Moreover, the relationship between this polymorphism and rejection episode outcome (transplant recovery vs failure) was not statistically significant (p=0.13). **Conclusion:** The results of the present study show no significant correlation between corneal graft rejection, rejection recovery and Fas -670A/G gene polymorphism.

Keywords: Corneal Transplantation, Fas Ligand, Rejection

INTRODUCTION

Corneal graft is the most successful type of organ transplantation in humans, which in the absence of HLA matching and immunosuppressive therapy has a high success rate of graft acceptance. In other words, the rate of rejection episodes is less than 33% in five years follow-up as shown by many previous studies (1-3).

Unlike the conjunctiva, cornea is known to be an immunologically inert site, since the generation of immune responsiveness to foreign antigens (including transplantation) is relatively suppressed.

Although anatomical barriers and soluble mediators have been implicated in immune privilege, it appears that the apoptotic cell death of Fas positive cells by tissue-associated CD95

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Ligand (Fas Ligand, FasL or CD178) is also important in corneal transplantation survival (2).
CD95 gene is located on chromosome 10q24 (3,4). It has been shown that different alleles can result in different binding capacities to transcription factors and therefore in different gene expressions (5,6).
In this respect, the presence of -670 A allele in the promoter region of Fas gene is associated with higher levels of gene expression (7). This fact has led to the growing idea that Fas ligand gene polymorphism may interfere with corneal transplantation outcome. Therefore, the aim of the present study was to investigate the association between functional -670 G/A Fas gene polymorphism and outcome of corneal transplantation.

MATERIALS AND METHODS

In this cross-sectional study, 276 corneal graft recipients who underwent penetrating keratoplasty (PK) for single eye disorder, such as aphakic bullous keratopathy, Pseudophakic bulbous keratopathy, corneal opacity, corneal dystrophy, keratoconus and corneal ectasia were included. Preoperative evaluations of patients and postoperative remarks such as graft status were obtained from their charts. Patients receiving PK because of inflammatory or infectious disorders or other underlying disorders, which made_them of high risk for graft rejection, such as preoperative corneal vascularization, were excluded (Table1). Moreover, patients with non-immunologic graft failure in their charts and no clinical sign of rejection were not included in this study. Graft rejection was determined as any of the signs of endothelial rejection such as keratic precipitate (KP) just behind the transplant, Khodadoust endothelial rejection line, anterior chamber reaction, localized or diffuse graft edema, demonstrated by clinical examination under slit lamp biomicroscpe, while epithelial or stromal rejection were not considered since these types of graft rejections would have different behaviour and better prognosis and usually would not lead to irreversible graft failure. In all patients with signs of endothelial rejection, treatment started with both frequent topical and systemic corticosteroids initially and then as clinical response was detected, the drugs were tapered off. In those patients who did not respond to the above regimen, immunosuppressant therapy with cyclosporine was added to other medications. Clinical improvements proved by slit lamp examination such as decrease in AC reaction or clearance of graft edema and disappearance of KP or endothelial rejection line were considered as graft recovery.

Table 1. Indications for corneal graft

Cases	Number	Percent
Keratoconus	137	50.2
Corneal opacity	66	24.2
Pseudophakic bullous Keratopathy	38	13.9
Aphakic bullous keratopathy	8	2.9
Fuch's endoth dystrophy	5	1.8
Congenital hereditary endothelial dystrophy	4	1.5
Pellucid Marginal degeneration	1	0.4
Corneal laceration	4	1.5
Macular dystrophy	5	1.8
Other corneal ectasia	2	0.7
Total	276	100

About 1ml of venous blood was obtained from each patient after receiving informed consent and preserved in oxalate tubes at - 60°C. Then genomic DNA was extracted from patients' peripheral blood leukocytes by a salting out procedure. The Fas -670 A/G polymorphism was detected by allele-specific oligonucleotide polymerase chain reaction (ASO-PCR). The sense primers specific for -670 A/G was 5'-TGAGAGGCTCACAGACGTT- 3' and the antisense primers were 5'-GTGCACAAGGCTGGCACG -3' and 5'- GTGCACAAGGCTGGCACA-3'. As an internal control, the β-globin specific primers were included in the polymerase chain reaction (ASO-PCR). For Fas genotyping, 50μL of PCR reaction mixture consisting of 50 ng of genomic DNA, 200μmol/L dNTPs, 2 mM magnesium chloride, IX Taq DNA polymerase buffer, 2 units Taq DNA polymerase (Bohringer Manheim, Germany), 10 pmol of each test primer and 5 pmol of internal control primers were employed. Cycling conditions were as follows: 95°C for 5 minutes; 31 cycles at 94°C for 25 seconds, 65°C for 50 seconds, and 72°C for 45 seconds; 72°C for 10 minutes.

Reaction products of Fas gene were separated on a 2.5% agarose gel and finally stained with ethidium bromide. Electrophoresis of PCR product for the Fas gene gave a single band of 764 bp and the internal control showed a band of 100 bp.

Statistical Analysis. Data were analyzed by Chi-square test and Fisher's exact test when appropriate. All tests were performed two tailed with a confidence interval (CI) of 95%. Statistical calculations were carried out using the Epi Info 2000 software. P-Values less than 0.05 were considered to be significant.

RESULTS

There were 276 patients including 118 (42.8%) females and 158 (57.2%) males with the mean age of 40.3 ± 1.3 years (range, 6-80 years).

Indication for PK in most cases were keratoconus (50.2%) followed by corneal opacity (24.2%), aphakic and pseudophakic bullous keratopathy (16.8%). Other less common underlying disorders are also demonstrated in Table 1. The mean follow up of precipitants of this study was 2.2 years +/- 0.23 (range: 2-5 years).

Considering graft status, in age matched groups with the mean age of 43.67 +/-22.18 and 40.08 +/- 22.18, respectively (p= 0.123), there were 47 (17%) patients who had experienced at least one episode of endothelial graft rejection in their follow up course, while 225 (83%) patients did not experience any such episode. According to FAS ligand gene polymorphism among patients with rejection, the frequency of AG was only 20% compared with AA and GG, which were 25.5% and 31.9%, respectively (Table 2). Therefore, there was no statistically significant relationship between Fas ligand polymorphism and occurrence of endothelial graft rejection in these patients (p=0.350). Similarly in patients without any previous rejections, the three types of gene polymorphisms showed equal distribution; 34.2 % of patients had AA polymorphism, 32.4 % AG and 33.3% GG (p=0.31). Moreover, the association between Fas -670 A/G polymorphism and the number of rejections was not also statistically significant (p=0.99).

However, the association between Fas gene -670 A/G polymorphism and rejection outcome (recovery vs failure) was statistically insignificant (p=0.13). In fact, as shown in Table 2, among carriers with -670 AA genotype, the percentage of patients who recovered from rejection were higher compared to patients who eventually rejected the graft (33.3% and 1.7%, respectively).

Table 2: Association between Fas -670 A/G polymorphism and rejection episode, Frequency of rejection episodes and rejection outcome

Rejection status		Fas- 670 *AIG* polymorphism			P-Values
		AA	AG	GG	
		N(%)	N(%)	N(%)	
Rejection	+	12(25.50)	20(42.5)	15(31.9)	0.35
	-	77(34.2)	73(32.4)	75(33.3)	
Number of rejection N(%)	No episode of rejection	77(34.2)	73(32.4)	75(33.3)	0.99
	One episode of rejection	11(33.5)	12(36.3)	10(30.3)	
	Two episode of rejection	1(33.3)	1(33.3)	1(33.3)	
Rejection	Recovery	11(33.3)	11(33.3)	11(33.3)	0.13
	No response to treatment	1(7.7)	8(61.5)	4(30.8)	

DISCUSSION

Corneal immune privilege is due to multiple factors. Among them, expression of Fas ligand by corneal cells is believed to play a critical role in maintenance of corneal transplants by inducing Fas-mediated apoptosis in activated lymphocytes. Therefore, high percentage of corneal transplants is accepted without tissue matching or immunosuppressive therapy (8). Fas molecule was proven to be important in cell death and apoptosis by previous investigators.

Physiological ligand for Fas molecule is FasL, which is a type II transmembrane protein expressed by activated T cells and non-T cells under a variety of conditions. The extracellular domain of FasL has the ability to bind Fas in the target cell. The interaction of Fas 48 KDa type I membrane protein that is expressed by many nucleated cells causes cross-linking of Fas and this in turn transduces an active signal for cellular apoptosis which is mediated through activation of a group of cytosolic enzymese called "caspases" (3,4).

The effect of Fas/Fas ligand system on organ transplantation of heart, liver and kidney was suggested. It was proposed that this system with its effect on T lymphocyte death had a strong effect on immune response to organ transplantation (8-10).

There is abundant FasL expression throughout the eye, including the iris, ciliary body and the cornea (5). In the cornea, fully functional FasL is expressed on the endothelium and epithelium and the importance of FasL expression by corneal cells in killing Fas+ lymphoid cells has been shown in in vitro culture systems. Histological analysis also showed that Fas Ligand positive (FasL+) grafts contained apoptotic mononuclear cells, while rejecting Fas ligand negative (FasL-) cornea contained numerous inflammatory cells without associated apoptosis (5). These studies suggest that activation of Fas/FasL system may abolish the process of graft rejection by killing Fas+ effector cells arriving in the graft from the lymphoid system (8).

Considering the importance of Fas/FasL interaction in graft survival, the aim of the present study was to investigate the association between Fas gene -670 A/G polymorphism with corneal allograft rejection. Other polymorphisms are presented also for this gene, which will be a subject of future studies. As was mentioned, our results showed that there was no statistically significant association between corneal endothelial rejection episode and Fas -670 A/G polymorphism. It is noteworthy to indicate that the absence of any significant association between rejection outcome and Fas -670 A/G polymorphism may be related to the presence of low number of rejections in the present study which was about 46 cases. Therefore, this correlation may differ if studies are performed on larger number of patients.

Considering the higher expression of Fas gene in the presence of -670 A allele (11,12), it could be proposed that activated T lymphocytes from -670 A carriers are more susceptible to apoptosis after Fas/FasL interactions. Therefore, after rejection episodes in patients with Fas -670 AA genotypes, the chance of graft failure due to elimination of activated alloreactive T lymphocytes is less than the carriers of G allele (A/G and G/G genotypes). As mentioned, both groups were age matched, with the same mean duration of follow up, so age and time did not affect our results. Moreover, we excluded all patients who were considered as high risk for graft rejection or poor follow up, eliminating some possible confounding factors as much as possible, however, the possible influence of some uncontrolled factors should be still considered as limitations of the present study.

The results of the current study were in favour of the absence of correlation between corneal endothelial allograft rejection and Fas gene polymorphism. Moreover, it was found that there was not a higher chance of medical recovery of the graft after rejection in AA variants of Fas ligand gene polymorphisms compared to other variants.

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