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Lack of Association Between TNF α -308 Polymorphism and End Stage Renal Disease in North Indian Population

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KEYWORDS TNF-α; ESRD; polymorphisms; SNP

ABSTRACT Cytokines, play a critical role in the pathophysiology of End Stage Renal Disease (ESRD). Tumor necrosis factor, TNF-α, is a multifunctional cytokine implicated in modulating the progression of ESRD. The promoter of TNF-α gene has many biallelic variations but the one at -308 (G-308 A) relative to transcription start site has extensively been studied and reported to have implications in acute and chronic rejection after renal transplant, the best renal replacement therapy available to ESRD patients. The present study was undertaken to evaluate the role of a single nucleotide polymorphism at -308 site in the promoter of TNF-α gene in ESRD in North Indian population. Samples from 111 diabetic nephropathy patients with ESRD and 164 random controls were genotyped using amplification refractory mutation system analysis for this polymorphism. The two groups revealed no significant differences with respect to the distribution of-308 polymorphic genotypes or allele frequencies. The data strongly suggest a lack of association between TNF-α-308 polymorphism and ESRD in the North Indian population and lend support to the argument that the association of A allele (high TNF-α producer) with transplant rejection may reside with the involvement of this allele in the process of rejection.

INTRODUCTION

ESRD is a term used for kidney failure patients who are permanently dependent upon renal replacement therapy (dialysis or transplantation) in order to avoid life-threatening uremia (Levey et al. 2003). In India, it is difficult to decipher the exact number of patients afflicted with this disease due to the lack of national registries; however, using most conservative estimates it is estimated that around 100,000 fresh patients are added each year to the existing pool of patients (Sakuja et al. 1994). Diabetic nephropathy and hypertension are two leading causes for the development of ESRD (Young 1997). Diabetic nephropathy is a common complication of diabetic mellitus and is shown to be influenced by genetic factors (Chaudhary et al. 1999). Several investigators have implicated tumor necrosis factor –alpha (TNF-α) cytokine in modulating the progression of ESRD (Sankaran et al. 1999; Klahr 1999). The promoter of TNF-α gene has many single nucleotide polymorphisms (SNP) but the one at -308 (G-308 A) relative to transcription start site has extensively been studied (Wilson et al. 1992; Wilson et al. 1997; Hutchinson et al. 1998). The in vitro and *in vivo* data strongly alludes to the association of A allele with its high production. Keeping in view, the extent of the ESRD disease and the huge cost incurred for renal transplantation, the best renal replacement therapy (RRT) available to ESRD patients, new strategies are urgently needed for the better management of this chronic disease. The present study was, thus, undertaken to evaluate the role of a SNP at -308 site in TNF-α promoter as a risk factor in the development of ESRD.

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MATERIAL AND METHOD

Peripheral blood samples were collected from 111 diabetic nephropathy patients with ESRD and 164, age and sex matched, normal controls inhabiting the North Indian state of Punjab and adjoining areas. DNA was isolated from blood samples using inorganic method (Miller et al. 1988). The TNF-α-308 polymorphisms was studied employing ARMS-PCR as described by Gupta and Sehajpal (2003). Briefly, the optimized reaction conditions consisted of 40 ng of genomic DNA in a total volume of 30 µl of reaction mixture containing 0.16 µM of each

primer, 30 µM of each dNTP, 10mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl , 0.01 % gelatin and 0.3 unit of Taq DNA polymerase (Bangalore Genei, Bangalore). The reaction was amplified for 35 cycles, each cycle consisted of denaturation at 94ºC for 30 sec, annealing at 57ºC for 20 sec, extension at 72ºC for 20 sec and finally a 3 min extension at 72ºC.

RESULTS AND DISCUSSION

Figure 1 represents a typical agarose gel of amplified products. Upper and lower panels of the gel show the PCR products amplified with primers AP1 (specific for *G* allele) & CP and AP2 (specific for *A* allele) & CP, respectively. Lanes 2-6 shows the randomly screened samples for determination of genotypes, represented at the bottom of the gel. Lanes 1 and 7 represent known control samples of GG and AA genotypes, respectively. It is evident from table 1 that the incidence of homozygote for *G* allele (GG) for this polymorphism were higher in the control group (84.15%) than the ESRD patients (82.88%), while the incidence of heterozygote (GA) (13.52%) and homozygote (AA) for *A* allele (3.60%) are marginally higher in ESRD group then the controls (13.41%) and (2.44%), respectively. No significant differences in the distribution of genotypes (χ^2 =0.321, p=0.1483, df=2) and polymorphic alleles was observed in diabetic nephropathy ESRD patients and control population.

Table 2 exhibits a wide variation (0.068-0.274) in the incidence of *A* allele in different populations of the world (Gupta and Sehajpal 2003; Kam et al. 1999; Witte et al. 2002; Westendorp et al. 1997; Schaaf et al. 2001; Lio et al. 2003; Perrey et al. 1998; Meenagh et al. 2002). It is interesting to note that the frequency of the *A* allele was found to be lowest (0.068) amongst the North Indian population of Punjab, followed by 0.091 in the present investigation. The highest frequency (0.274) was recorded in a Caucasian population from Manchester (UK).

ESRD is a human clinical disorder where renal transplantation is the best possible therapy. Keeping in view the Indian population structure and its gross GDP value, it won't be unfair to presume that a large number of patients afflicted with this disease die as they cannot afford either renal transplantation or renal dialysis for long duration of time due to the lack of adequate funds to support these clinical procedures. Under such circumstances, it would be prudent to identify

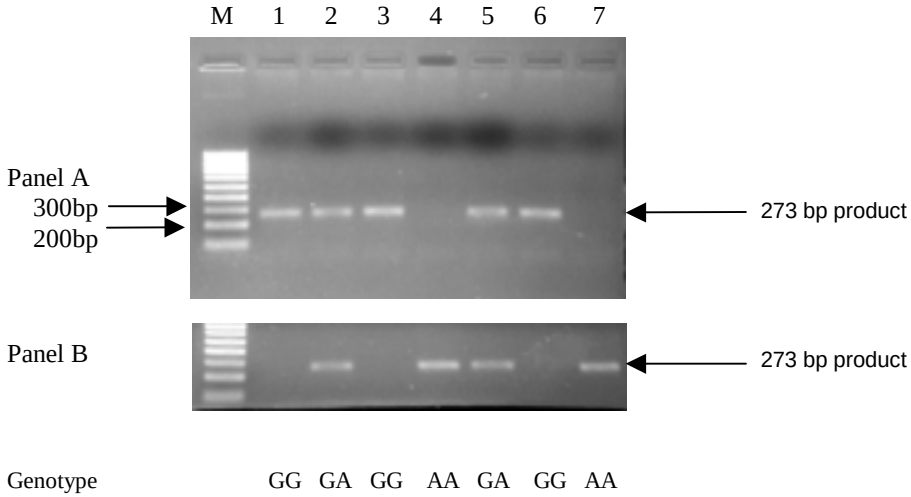

Fig. 1. A representative agarose gel photograph of the G-308A TNF-α polymorphism. The panels A and B depict the DNA samples amplified with AP-1(:5' ATAGGTTTTGAGGGGCATCG 3') & CP (CP: 5' AAGAATCATTCAACCAGCGG 3') and AP-2 (5' ATAGGTTTTGAGGGGCATCA 3') & CP, primer pairs, respectively. Lanes M represents 100 bp ladder. Lane 1 and 7 represents known samples of GG and AA genotypes, respectively. Lane 2-6 corresponds to random samples screened for -308 polymorphism. The genotypes of the samples are noted at the bottom of the figure.

Table 1: Genotypic distribution of TNF-α-308 polymorphism in control and ESRD populations

Population studied	*Genotypes (Percentage)*			*Allele frequency*	
	GG	*GA*	*AA*	*G*	*A*
ESRD (n=111)	92 (82.88)	15 (13.52)	4 (3.60)	0.896	0.104
Control (n=164)	138 (84.15)	22 (13.41)	4 (2.44)	0.909	0.091

Chi square (ESRD vs control) d.f. 2 = 0.321

Table 2: Genotypic and allelic distribution of TNF-α-308 polymorphism in different populations

Population studied	*Genotypes number (Percentage)*			*Allele frequency*		*Reference*
	GG	*GA*	*AA*	*G*	*A*	
North India, India	138 (84.2)	22 (13.4)	4 (2.4)	0.909	0.091	Present study
Punjab, India	165 (86.8)	24 (12.6)	1 (0.5)	0.932	0.068	Gupta and Sehajpal (2003)
Nottingham, England	96 (62.7)	52 (33.9)	5 (3.2)	0.797	0.202	Kam et al. (1999)
California, USA	212 (78.0)	55 (20.0)	6 (2.0)	0.877	0.122	Witte et al. (2002)
Leiden, Netherlands	50 (57.0)	34 (34.0)	4 (2.0)	0.761	0.238	Westendorp et al. (1997)
Kiel, Germany	143 (66.2)	63 (29.6)	10 (4.6)	0.81	0.19	Schaaf et al. (2001)
Central and Southern Italy	83 (72.2)	31 (26.9)	1 (0.8)	0.857	0.143	Lio et al. (2003)
Manchester, UK	65 (61.3)	24 (22.6)	17 (16.0)	0.726	0.274	Perry et al. (1998)
N. Ireland, UK	61 (61.0)	32 (32.0)	7 (7.0)	0.77	0.23	Meenagh et al. (2002)
Natal, (Zulu), South Africa	54 (62.8)	26 (30.2)	6 (7.0)	0.779	0.221	Meenagh et al. (2002)
Singapore, Chinese	64 (77.1)	18 (21.7)	1 (1.2)	0.88	0.12	Meenagh et al. (2002)

strategies that could help in the early identification of patients with high risk of developing ESRD or to look for ways that could delay the onset of this disease or modulate its course of infection.

Cytokines play a critical role in the pathophysiology of ESRD. Johnston et al. (1998) observed increased turn over of various cytokines in ESRD and suggested that genetic factors play a significant role in susceptibility of this disease in patients of African descent. Hutchinson et al. (1998) demonstrated that individuals differ in their capacity to synthesize various cytokines and attributed such differences to the existence of allelic polymorphism in the promoter region of cytokines. Tumor necrosis factor-α is a multifunctional cytokine implicated in modulating the progression of chronic renal failure to ESRD (Sankaran et al. 1999; Klahr 1999). Studies form different parts of the world suggest the association of A allele, high TNF-α producer, with acute rejection of renal transplant (Hassan et al. 2003; Ram et al. 2003). Although, this observation is not shared by all, Marshall et al. (2000) failed to find such association with renal graft rejection in their population. The present study was undertaken to investigate the association of TNF-α -308 allelic polymorphism and ESRD as no such data is available for Indian populations. The data obtained using 111 diabetic nephropathy ESRD patients and 164 controls clearly suggest a lack of association between TNF-α-308 polymorphism and ESRD in our population (chi-square value=0.321, df=2). These findings are in accordance with an earlier report (Poli et al. 2000) and lend support to the argument that the association of A allele with transplant rejection may reside with the involvement of this allele in the process of rejection. Given the wide variation observed in the incidence of this allele, it seems plausible that ethnic background of the population could as well play a role in this process. In conclusion, the result of this study indicates that TNF-α-308 promoter polymorphisms in not associated with ESRD in our population.

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