Recurrent pregnancy loss: TNF-α and IL-10 polymorphisms

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ABSTRACT

BACKGROUND: The recurrent pregnancy loss requires careful consideration of genetic, anatomic, endocrine, infectious and immunological factors. Cytokine gene polymorphisms in the promoter regions of tumor necrosis factor (TNF)-α and interleukin (IL)-10 are associated with recurrent pregnancy loss. **AIM:** The aim of present study was to investigate the association of the IL-10 -592C/A and TNF-α-308 G/A, promoter polymorphisms among women with at least three consecutive miscarriages. **MATERIALS AND METHODS:** Genotyping was done in 50 women with RPL for IL-10-592C/A and TNF-α-308G/A promoter polymorphism to see the association of these loci with pregnancy loss. The control group included 50 healthy women having two or more children (mean age of the female subjects 35 years) for statistical comparisons. **RESULTS:** IL-10-592C/A and TNF-α-308G/A promoter polymorphisms were not associated with the recurrent miscarriages. **CONCLUSIONS:** There is a need to screen a larger sample and in different ethnic groups using IL-10-592C/A and TNF-α-308G/A markers to understand their association with recurrent miscarriages. This would further help in efficient management of immunologically mediated recurrent miscarriages at the sample/individual level.

KEY WORDS: Cytogenetic, cytokines, IL-10, polymorphism, recurrent, TNF-α

INTRODUCTION

Recurrent pregnancy loss (RPL) can be defined as occurrence of three or more clinically detectable pregnancy losses. RPL can be caused by genetic, endocrine, anatomic, immunologic factors, and so on.[1,2] Despite several well-established etiologic factors, the cause of RPL cannot be determined in almost 50% of cases. Cytokines represent immunomodulatory proteins and are particularly important for both innate and adaptive immune responses. Depending on their inflammatory reactions, cytokines are broadly categorized into pro-inflammatory and anti-inflammatory cytokines produced by Th1 and Th2 cells, respectively. Th1 and Th2 cells reciprocally regulate each other's function through their respective cytokines.[3,4] Th2 immune reactions support normal pregnancy, while Th1 immunity is considered detrimental to the fetus.[5] The chromosomal location of the gene controlling the secretion of tumor necrosis factor (TNF)-α (Th1) is 6p21.3. Some of the polymorphisms that have been reported in this gene are −238; −308; and −863 located in the promoter region, and especially −308 G/A is known to cause an altered promoter activity, resulting in an increased production of TNF-α cytokine in blood.[6] Certain cytokine gene polymorphisms influence the level of cytokine production and associated with susceptibility to diseases and/or different clinical features/outcomes of diseases.[7] IL-10 plays a key role in Th2 immunity and is located on human chromosome 1 (1q31–q32). Many single-nucleotide polymorphisms (SNPs) are reported in the proximal (−1082A/G; -819T/C; −592A/C) and distal region of the IL-10 gene involved in IL-10 transcription rate and affecting its production level.[8-10]

We investigated −592C/A in IL-10 and −308G/A in TNF-α promoter gene polymorphisms in view of their critical role in regulation, balance, and maintenance of successful pregnancy.

MATERIALS AND METHODS

For the present study, 50 women with more

than 3 miscarriages were chosen for the molecular study. Informed consent was obtained from all the subjects to carry out cytogenetic/molecular analyses, and approval of institutional ethical committee was obtained. Detailed pedigree analyses and in-depth evaluation of the clinical reports was undertaken in all the subjects. Patients having anatomical abnormalities, acquired or hereditary thrombosis, hormonal disorders, that is, abnormal thyroid function, hyperprolactinemia, erythroblastosis fetalis (Rh disease), chromosomal aberrations, and Toxoplasmosis, Other infections, Rubella, Cytomegalovirus, Herpes simplex virus (TORCH) infections were excluded from the molecular study. The control group included 50 healthy women having two or more children (mean age of the female subjects 35 years) for statistical comparisons.

Total genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood leukocytes by phenol extraction method[11] with modifications to carry out polymerase chain reaction (PCR)-based analysis for the polymorphism in IL-10-592C/A and TNF-α-308 G/A followed by Restriction fragment length polymorphism (RFLP) analyses. The DNA samples were amplified for IL-10-592C/A and TNF-α-308G/A polymorphism by using specific primers[6] [Table 1]. The PCR products were electrophoresed on 2% agarose gel. The electrophoresis was undertaken at 50 V for 1.5 h and gel viewed under UV trans-illuminator. PCR products of promoter polymorphisms for IL-10-592C/A (258 bp) was digested with *RsaI* and for TNF-α-308G/A, (107 bp) was digested with *NcoI* restriction enzyme, respectively. For IL-10-592C/A, 258 bp fragment represented CC homozygote, 258; 221, 37 bp C/A heterozygote; 37 bp AA genotypes. For TNF-α-308G/A promoter polymorphism, GG homozygous genotype was represented by 87bp, G/A by 87 and 20bp; 107bp and AA genotype by 107 bp fragments, respectively.

Table 1: List of primers

	Primer sequence	PCR product size (bp)
Primers for IL-10-592C/A		
Forward primer	5′–TGTGCCTCAGTTTGCTCA-3′	250
Reverse primer	5′–CTTCCATTTACTTTCCAGAGACT-3′	
Primers for TNF-α-308G/A		
Forward primer	5′–AGGCAATAGGTTTTGAGGGCCAT-3′	107
Reverse primer	5′-TCCTCCCTGCTCCGATTCCG-3′	

Statistical analysis

Allele frequencies were calculated for each genotype and the difference in allele frequencies between recurrent miscarriage cases and control women having 2 or more children were determined by using a Pearson χ^2 test (SPSS Inc.10, Chicago, IL, USA). Expected genotype frequencies were calculated from the allele frequencies under the assumption of Hardy–Weinberg equilibrium.

RESULTS

Fifty cases of RPL and 50 healthy control women with two or more children were genotyped for the IL-10-592C/A and TNF-α-308G/A promoter polymorphisms to find the association of these genotypes or alleles with recurrent miscarriages. The age range of study sample was from 20 to 35 years. The mean maternal age was 25.9 years and that of controls was 26.4 years. The mean miscarriages were 3.39. Mean gestational age was 3.6 months. Patients having anatomical abnormalities, acquired or hereditary thrombosis, hormonal disorders, chromosomal aberrations and TORCH infections were not included in genotyping.

IL-10-592C/A polymorphism analyses

Out of 50 cases in recurrent miscarriages for IL-10-592C/A promoter polymorphism, the genotype frequencies in IL-10-592C/A promoter polymorphism were found to be 0.36, 0.48, and 0.16 for CC, CA, AA, genotypes, respectively, in cases with recurrent miscarriages whereas those for normal control were calculated to be 0.34, 0.54, and 0.12 for CC, CA, and AA, respectively. The allele frequency for C in affected and control cases was 0.60 and 0.61, respectively, while that of A allele was 0.40 and 0.39. The frequencies do not show preferential prevalence of a particular allele in a particular group and thus there was no significant statistical difference in genotype distribution and allele frequency between recurrent miscarriage cases and controls. Allele and genotype frequencies are indicated in Table 2. IL-10-592C/A promoter polymorphism in the present case–control study does not show any association with the recurrent miscarriages (Chi-square value for genotypes = 0.491 and for alleles = 0.021 and $P > 0.05$).

TNF-α-308G/A polymorphism analyses

Out of 50 cases of RPL, the genotype frequencies for GG, GA, and AA in TNF-α-308G/A promoter polymorphism were 0.78, 0.12, and 0.10, respectively, whereas for normal controls 0.82, 0.14, and 0.04 for CC, CA, and AA, respectively. The observed allele frequency for G allele in RPL cases and controls was 0.84 and 0.89 and that of A was 0.16 and 0.11, respectively [Table 2]. There was no preferential prevalence of a particular allele, and thus no statistically significant difference in genotype distribution of allele frequency in recurrent miscarriage cases and controls. The allele and genotype frequencies are shown

Table 2: Allele and genotype distribution of IL-10-592C/A and TNF-α-308 G/A

	Genotypes			Alleles	
IL-10-592C/A	C/C	C/A	A/A	C	A
RPL cases (50) (%)	18 (36)	24 (48)	8 (16)	0.60 (60)	0.40 (40)
Controls (n = 50) (%)	17 (34)	27 (54)	6 (12)	0.61 (61)	0.39 (39)
χ^2		0.491		0.021	
P value		0.782		0.885	
TNF-α-308 G/A	G/G	G/A	A/A	G	A
RPL cases (50) (%)	39 (78)	6 (12)	5 (10)	0.84 (84)	0.16 (16)
Controls (n = 50) (%)	41 (82)	7 (14)	2 (4)	0.89 (89)	0.1111
χ^2		1.413		1.070	
P value		0.493		0.301	

IL - Interleukin-10, TNF - Tumor necrosis factor, RPL - Recurrent pregnancy loss

in Table 2. Statistical analysis revealed that TNF-α-308G/A promoter polymorphism in the present sample is not associated with recurrent miscarriages (Chi-square value for genotypes = 1.413 and for alleles = 1.070 and $P > 0.05$).

DISCUSSION

The evaluation of RPL cases requires careful consideration of genetic, anatomic, endocrine, infectious, and immunologic factors. The molecular study was undertaken on 50 RPL cases and 50 healthy control women. Genotyping was done for IL-10-592C/A and TNF-α-308G/A promoter polymorphism to see the association of these loci with miscarriages, if any and thus to correlate susceptibility to recurrent pregnancy loss. This was a case–control study where comparison was made of the genotype and allele frequencies between the women with recurrent miscarriages and controls in a statistical manner. The results indicated absence of any association of a particular genotype or allele of IL-10-592C/A and TNF-α-308G/A promoter polymorphism with the recurrent miscarriages. The values indicated higher frequencies of CA genotype (48%) among RPL cases and 54% in control cases in IL-10-592C/A promoter polymorphism. This difference was not well marked and was statistically nonsignificant. The frequency of AA genotype was 16% in RPL cases and 12% in healthy control women was also statistically nonsignificant. Statistical analysis revealed that IL-10-592C/A promoter polymorphism in the present sample does not show any association with the recurrent miscarriages (calculated Chi-square value for genotypes = 0.491 and for alleles = 0.021). Our results are consistent with Costeas *et al.*,[12] Bombell and McGuire,[13] and Prigoshin *et al.*[14] However, the findings of the present study are in contradiction to reports by Kamali-Sarvestani *et al.*[15] Their results indicated a significant association between the presence of CC genotype of IL-10-592C/A polymorphism and the occurrence of RPL in Iranian women (63% in women with RPL and 46% in controls).

The allele and genotype frequencies were studied in TNF-α-308G/A promoter polymorphism to find any association of a particular genotype or allele with the recurrent miscarriages. The results indicated the absence of any association of a particular genotype or allele with recurrent miscarriages. The distribution indicated higher frequency of GG genotype among both the RPL cases (78%) and normal controls (82%) in TNF-α-308G/A promoter polymorphism and the difference was statistically nonsignificant. Statistical analysis revealed that TNF-α-308G/A promoter polymorphism in the present sample does not show any association with recurrent miscarriages (calculated Chi-square value for genotypes = 1.413 and for alleles = 1.070). Our results are consistent with those reported by Babbage *et al.*,[16] Baxter *et al.*,[17] Pietrowski *et al.*,[18] Prigoshin *et al.*,[14] Kamali-Sarvestani *et al.*,[15] Bombell and McGuire,[13] Menon *et al.*,[19] and Zammiti *et al.*,[20] However, the findings of the present study are in contradiction to reports by Daher *et al.*,[21] and Costeas *et al.*[12] These studies provided evidence for the association of AA genotype with recurrent miscarriages.

The absence of any positive association in the present study may be due to small sample size. The selected molecular markers, that is, IL-10-592C/A and TNF-α-308G/A are the candidate genes for the recurrent miscarriages and the manifestation of the disease vary in different ethnic groups as they are largely influenced by the mating patterns, surrounding genetic environment, lifestyle factors, and other environmental factors, which are sample specific.

In conclusion, there is no association of IL-10-592C/A and TNF-α-308G/A promoter polymorphisms with recurrent pregnancy loss in the present investigated sample. There is a need to screen larger sample and in different ethnic groups to understand the extent of association between RPL and these molecular markers. It would further help in bringing out the community-specific associations and efficient management of the immunological factors in recurrent miscarriages at the sample/individual level.

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