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

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.

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 were undertaken in all the subjects. Patients having anatomical abnormalities, acquired or hereditary thrombosis, hormonal disorders, that is, abnormal thyroid function, hyperprolactinemia, erythroleukemia 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[1][1] 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 Rsal and for TNF-α-308G/A, (107 bp) was digested with Ncol 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) |
|-----------------|---------------------|
| **Primer for IL-10-592C/A** | |
| Forward primer | 5′–TGTGCTCAGTGTGCTCA-3′ |
| Reverse primer | 5′–CTTCCATTACCTTCCAGACT-3′ |
| **Primer for TNF-α-308G/A** | |
| Forward primer | 5′–AGGCATAAGGTTTGAGGGCCAT-3′ |
| Reverse primer | 5′–TCCCTCGTCTCCGATTCCG-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 χ² 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 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

92

Journal of Human Reproductive Sciences / Volume 4 / Issue 2 / May - Aug 2011
Kaur and Kaur: Recurrent pregnancy loss: TNF-α and IL-10 polymorphisms
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 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.,[15] 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.

**REFERENCES**

1. Stirrat GM. Recurrent miscarriage; definition and epidemiology. Lancet 1990;336:1402-6.
2. Bricker L, Farquharson RG. Types of pregnancy loss in recurrent miscarriages.
Cytokine gene polymorphism in human disease: On line

Only the references from journals indexed in PubMed will be checked.
Add up to a maximum of 15 references at a time.
Enter each reference in new line, without a serial number.

Example of a correct style

The style as well as bibliographic elements should be 100% accurate, to help get the references verified from the system. Even a

Lack of association of TNF alpha gene polymorphisms and idiopathic recurrent miscarriage. J Reprod Immunol 2005:65:171-8.

Babbage SJ, Arkwright PD, Vince GS, Perrey C, Pravica V, Quenby S, et al. Cytokine promoter gene polymorphisms and idiopathic recurrent miscarriage. J Reprod Immunol 2001:126:529-34.

Pietrowski D, Bettsendorf H, Keck C, Bürkle B, Unfried G, Rienek EK, et al. Lack of association of TNF alpha gene polymorphisms and recurrent pregnancy loss in Caucasian women. J Reprod Immunol 2004:61:51-8.

Menon R, Merialdi M, Betrán AP, Dolan S, Jiang L, Fortunato SJ, et al. Analysis of association between maternal tumor necrosis factor-alpha promoter polymorphism (-308), tumor necrosis factor concentration, and preterm birth. Am J Obstet Gynecol 2006:195:1240-8.

Zammiti W, Mitraniou N, Khairi H, Gris JC, Almawi WY, Mahjoub T. Associations between tumor necrosis factor-alpha and lymphotixin-alpha polymorphisms and idiopathic recurrent miscarriage. Reproduction 2008:135:397-3.

Daher S, Shulzenko N, Morgun A, Mattar R, Rampim GF, Camano L, et al. Associations between cytokine gene polymorphisms and recurrent pregnancy loss. J Reprod Immunol 2003:58:69-77.

How to cite this article: Kaur A, Kaur A. Recurrent pregnancy loss: TNF-α and IL-10 polymorphisms. J Hum Reprod Sci 2011;4:91-4.

Source of Support: This work was supported by GNDU grant [Synd.-27(28/6/07)] to AK.

Conflict of Interest: None declared.

Author Help: Reference checking facility

The manuscript system (www.journalonweb.com) allows the authors to check and verify the accuracy and style of references. The tool checks the references with PubMed as per a predefined style. Authors are encouraged to use this facility, before submitting articles to the journal.

- The style as well as bibliographic elements should be 100% accurate, to help get the references verified from the system. Even a single spelling error or addition of issue number/month of publication will lead to an error when verifying the reference.

- Example of a correct style

Sheahan R, O’Leary G, Lee G, Fitzgibbon J. Cystic cervical metastases: Incidence and diagnosis using fine needle aspiration biopsy. Otolaryngol Head Neck Surg 2002:127:294-8.

- Only the references from journals indexed in PubMed will be checked.

- Enter each reference in new line, without a serial number.

- Add up to a maximum of 15 references at a time.

- If the reference is correct for its bibliographic elements and punctuations, it will be shown as CORRECT and a link to the correct article in PubMed will be given.

- If any of the bibliographic elements are missing, incorrect or extra (such as issue number), it will be shown as INCORRECT and link to possible articles in PubMed will be given.

94

Journal of Human Reproductive Sciences / Volume 4 / Issue 2 / May - Aug 2011