Original Article

Impact of Interleukin-10 Promoter Region Polymorphisms on Recurrent Miscarriage: A Case–Control Approach

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Abstract

Background: Recurrent miscarriage (RM), defined as two or more consecutive miscarriages prior to the 20th week of gestation is characterised by multifactorial aetiology. The prevalence of RM varies from 0.8% to 13.5% amongst women of reproductive age. The aetiological basis of RM has been traced to chromosomal, anatomic, hormonal and immunologic factors while half of the cases remain idiopathic. **Aims:** This study aimed to investigate the association of interleukin-10 (IL-10) polymorphisms with RM amongst the Indian population. **Settings and Design:** The present study included a total of 414 individuals including RM women (n = 199) with two or more pregnancy losses and healthy women (n = 215) without any previous history of pregnancy loss were taken as the control group. **Materials and Methods:** Demographic features and reproductive history of women with RM and healthy women were taken. Genotype analysis of IL-10 polymorphisms rs1800872 and rs1800896 was performed using the polymerase chain reaction (PCR) restriction fragment length polymorphism and amplification mutation refractory system PCR, respectively. **Statistical Analysis Used:** Student's t-test was used to compare the demographic features and reproductive history amongst both groups. Pearson's Chi-square was used to calculate the Hardy–Weinberg equilibrium, allelic and genotypic frequencies. All the statistical analyses were performed using the SPSS (version 21, IBM SPSS, NY, USA). **Results:** Our results suggested that the genotypic and allelic frequency of rs1800872 polymorphism did not differ significantly between RM cases and control women (P = 0.07 and P = 0.23, respectively). The GG genotype (P = 0.007) and G allele (P = 0.003) of rs1800896 were significantly associated with an increased risk of RM. A statistically significant difference was also found for the distribution of genetic models (dominant and co-dominant model) between both groups for rs1800896. However, haplotype analysis revealed that none of the haplotypes provides a risk for the progression of RM. **Conclusion:** The study is the first of its kind from our region and provides baseline data on the genetics of RM.

Keywords: *Cytokine, interleukin-10, polymorphisms, recurrent miscarriage*

Introduction

Recurrent miscarriage (RM) is defined as the loss of two or more consecutive pregnancies by ESHRE[1] and the American Society for Reproductive Medicine in the USA.[2] However, the previous definition of three or more consecutive pregnancy losses is still used by other organisations, including the RCOG (2011)

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and the Health Service Executive (HSE) in Ireland (HSE, 2016).[3] The prevalence of RM is claimed to range from 0.8% to 13.5% in women of reproductive age.[4-6] It can be classified into two types: primary and secondary. Primary RM describes pregnancy loss in women who never gave birth to a living child. While secondary RM is characterised as pregnancy loss in women who have already given birth to a living child.[7] There is no established cause of RM, however, a number of theories point to the involvement of chromosomal abnormalities, infections, antiphospholipid syndrome, thrombophilias, genetic factors, abnormalities of the uterus' anatomic structure and exposure to environmental variables.[8,9] Idiopathic causes account for about 50% of occurrences of RM.[10]

For a successful pregnancy, the induction of maternal tolerance to foreign foetal tissues, as well as a distinct cytokine production profile that promotes placental development and foetal growth is necessary. It has been demonstrated that decidual cells surrounding the early conceptus inhibit maternal immunity, thereby protecting the allogeneic foetus from infiltrating cytotoxic T-lymphocytes. According to a Th1/Th2 paradigm, early implantation is thought to require a Th1-type response mediated by interleukin (IL)-2, IL-1, whereas pregnancy maintenance depends on the contribution of regulatory (Treg) or anti-inflammatory (Th2-type) cytokines, in particular IL-10.[11] T-lymphocytes are essential for cell-mediated immunity, which plays a crucial role in pregnancy and miscarriage. At the foetal–maternal interface, several cytokines maintain the delicate balance required for the gestational period to be completed. If this complex network has dysregulated, immune-regulatory mechanisms could not be able to maintain the interface's homeostasis, which will lead to pregnancy failure.[12] It was reported that there was a decrease in the production of Th2, or anti-inflammatory cytokines, and an increase in proinflammatory cytokines (Th1) in women who experienced RM.[13,14]

Polymorphisms in cytokine genes may have an impact on both the immunological response induced and the expression of cytokines. Therefore, it is possible that cytokine gene polymorphisms may affect the likelihood of RM.[12] Human IL-10, which has a variable immunologic function that can be either stimulatory or counter-regulatory, is situated on chromosome 1q32. IL-10 is referred to as a Th2 cytokine and has anti-inflammatory effects against the cytokines produced by the Th1 subset. According to reports, reduced IL-10 levels and premature birth have also been linked.[15] The regulation of IL-10 levels may be affected by the polymorphisms in the *IL-10* promoter region.[15] Only a few studies have been conducted in India on the relationship between RM and IL-10 polymorphisms. Hence, the present case–control study focused on the analysis of the association of 1L-10 promoter region polymorphisms with RM.

Materials and Methods

Sample collection

In the present study, a total of 414 women comprising 199 women with clinical history RM were recruited as cases and 215 women of reproductive age, with at least one live birth and no miscarriage, were enrolled as controls. Women with cytogenetic abnormalities,[16] endocrinal disorders, hepatitis B, torch infections, autoimmune diseases and any positivity for anatomical anomalies as detected by ultrasonography were excluded from the study. The study was approved by the Ethical Review Board of Guru Nanak Dev University, (107/HG) and was in accordance with the Declaration of Helsinki of 1964 and its further amendments. All the RM cases were recruited from Beri Hospital and and Hartej Hospital. The individuals' complete information, including their demographics, menstrual and reproductive histories, family histories and pedigree, were recorded on a pre-designed pro forma. After obtaining written informed consent, about 5 ml of intravenous blood was withdrawn with a sterile syringe from each enrolled individual and poured into vials containing 0.5M EDTA for molecular genetic analysis. The samples were brought to the laboratory in an insulated box with frozen gel packs and were kept at −20°C till further analysis.

DNA isolation

Isolation of DNA was done from 3 ml of 0.5M EDTA blood by phenol extraction method as described by Adeli and Ogbonna with lab modifications[17] and quantified using NanoDrop™ 2000/200c spectrophotometer (Thermo Scientific TM, Pittsburgh, USA).

Genotypic analysis

For genotypic analysis, the polymerase chain reaction (PCR) restriction fragment length polymorphism and amplification mutation refractory system (ARMS) PCR techniques were used. The region of IL-10 containing rs1800872 and rs1800896 polymorphisms was amplified using the specific primers [Table 1] and PCR conditions used for amplifications are given in Table 2. For rs1800872, amplification followed by restriction digestion using *RsaI* (New England Biolabs) enzyme was done at 37°C for 2 h and then electrophoresed on 3.5% agarose gel. After digestion, a product of 258 bp represented the homozygous wild-type genotype, bands of 258 bp, 221 bp and 37 bp signified heterozygous

Table 1: Representation of Primer sequence and Size of amplicon

Polymorphisms	Primer Set	Primer Sequence (5' to 3')	PCR product size	Reference
rs1800872	Forward	TGTGCCTCAGTTTGCTCA	258	Kaur and Kaur[70]
	Reverse	CTTCCATTTACTTTCCAGAGACT		
rs1800896	Forward	CTTCCATTTACTTTCCAGAGACT		
	Forward	ACTACTAAGGCTTCTTTGGGAA-Y	248	Perrey *et al.*[71]
	Reverse	CTACTAAGGCTTCTTTGGGAG		

PCR=Polymerase chain reaction

Table 2: Amplification conditions for interleukin-10 (rs1800872 and rs1800896) polymorphisms

Serial number	Steps	rs1800872		rs1800896	
		Temperature (°C)	Time	Temperature (°C)	Time
1	Initial denaturation	95	5 min	95	1 min
2a	Denaturation	95	45 s	95	15 s
b	Annealing	62	30 s	65	50 s
c	Extension	72	45 s	72	25 s
		Steps 2a–2c repeated for 5 cycles		Steps 2a–2c repeated for 10 cycles	
3a	Denaturation	95	45 s	95	15 s
b	Annealing	61	50 s	61	50 s
c	Extension	72	45 s	72	45 s
		Steps 3a–3c repeated for 5 cycles		Steps 3a–3c repeated for 10 cycles	
4a	Denaturation	95	30 s	95	30 s
b	Annealing	59	45 s	61	50 s
c	Extension	72	45 s	72	50 s
		Steps 4a–4c repeated for 30 cycles		Steps 4a–4c repeated for 20 cycles	
5	Final extension	72	10 min	72	10 min
6	Hold	4	Infinity	4	Infinity

genotype and bands of 221 bp and 37 bp represented the homozygous mutant genotype. In the studied population the frequency of allele (A) was found to be lower as compared to the (G) allele.

Using an ARMS PCR method rs1800896 was genotyped. Allele-specific forward primers and a common reverse primer were used to amplify the region and then electrophoresed on ethidium bromide-stained 2% gel.

Statistical analysis

The sample size was calculated with the CaTS power calculator (http://www.sph.umich.edu/csg/abecasis/CaTS/index.html) for the present case–control study. An estimation of the effective sample size revealed a total of 180 cases and 180 controls with a power of the study 87%. Therefore, the sample size in this stage; 180 × 1.10 = 198 for cases and controls. It is believed that the present sample size (cases: 199; controls: 215, total = 414) with all aspects would be sufficient for the present case–control study for the molecular analysis. To calculate the distribution of allelic and genotypic frequencies and the Hardy–Weinberg equilibrium (HWE) of each polymorphism, the Chi-square test of Pearson was used. Clinical features of both groups were compared using Student's *t*-test. ANOVA test with a post-Tukey test was used to compare the mean age of cases and number of miscarriages. The odds ratios (ORs) were calculated with MedCalc statistical software and $P < 0.05$ was considered statically significant.

In association studies, the genetic model will improve the ability to identify risks associated with allelic variation of the candidate gene. Thus, different genetic models through linear regression analysis were constructed to check any disease risk association toward RM. All the statistical analyses were performed using the SPSS (version 21, IBM SPSS, NY, USA).

Results

The mean age of females with RM was 29.507 ± 3.71 years (range 20–42 years) and of healthy women was 29.865 ± 4.78 (range 21–44) years of age and remains non-significant amongst both groups ($P = 0.38$). Amongst 199 women with RM, 50.67% belonged to urban areas and 49.33% to rural areas. Similarly, 59.65% of control females were urban residents while 40.47% were rural inhabitants. The women with RM and control were matched for the dietary patterns ($P = 0.08$). None of the females in the case group was taking alcohol, while only one female in the control group was consuming alcohol occasionally. The females amongst both groups (cases with RM and controls) were non-smokers. Age at

menarche was observed to be a statistically significant difference (P = 0.001) between cases and controls. In RM cases, the mean age of women at the time of marriage was 25.218 ± 3.073 and in the control group was 23.190 ± 2.655. The mean age of women with RM at the time of first gravida was 25.870 ± 2.893 and in controls was 24.288 ± 2.563 and this difference is statistically significant (P < 0.001). A statistically significant difference (P = 0.0001) was observed between the mean gestational age of women with RM and control women with the mean gestational age of 10.434 ± 4.461 and 37.851 ± 0.646, respectively. However, body mass index (BMI) remains non-significant between RM cases and controls with P = 0.69 [Table 3]. The mean age of women was correlated with the number of miscarriages and it was found to be statistically significant. Miscarriages occur more in women of advanced age. However, the present study demonstrated that menarche age had no impact on number of miscarriages [Table 4]. When the distribution of RM women with respect to the number of miscarriages and age was done, it was found that the maximum number of women (51.2%) belonged to the age group of 25–29 years, around 11% of females were of advanced age in the study [Table 5].

Table 3: Characteristics features of recurrent miscarriage and healthy control women

Variables	Cases (n=199)	Controls (n=215)	P
Age of females	29.5±3.7	29.8±4.7	0.383
Age at menarche	13.17±1.1	13.5±1.0	0.001**
Age at marriage	25.21±3	23.1±2.6	0.000***
Duration of marriage	4.34±3.2	6.6±4.1	0.000***
Habitat, *n* (%)			
Urban	101 (50.67)	128 (59.53)	0.07
Rural	98 (49.33)	87 (40.47)	
Dietary pattern, *n* (%)			
Veg	164 (82.5)	191 (88.8)	0.08
Non-veg	35 (17.5)	24 (11.2)	
Alcohol consumption, *n* (%)			
Yes	0	1 (0.5)	-
No	199 (100)	214 (99.5)	
Use of contraceptives, *n* (%)			
Yes	13 (6.73)	75 (34.8)	0.000***
No	186 (93.27)	140 (65.1)	
Age at first gravida	25.8±2.8	23.7±2.5	0.000***
Gravida			
<3	95	211	0.000***
≥3	104	4	
Parity			
<2	223	19	-
≥2	0	196	
Mean gestational age	10.3±4.3	38±0.3	0.000***
BMI, *n* (%)			
Normal (18.5–22.9)	78 (39.4)	83 (38.6)	0.69
Underweight (≤18.5)	12 (5.8)	17 (7.9)	
Overweight/obese (≥23.0)	109 (54.7)	115 (53.4)	

**P<0.01, ***P<0.0001. BMI=Body mass index

Table 4: Comparison of the number of miscarriages as a function of age and age at menarche

	Number of miscarriages			P
	2M (n=105)	3M (n=66)	>3M (n=28)	
Mean age of women	28.9±3.5	29.7±3.5	30.9±4.1	0.02*
Mean age of women at menarche	13.228±1.236	13.094±1.100	13.129±1.024	0.723

*P<0.05, ANOVA analysis followed by *post-hoc* Tukey test. Statistically significant. M=Miscarriages

Molecular analysis

All the controls were subjected to HWE for both single nucleotide polymorphisms (SNPs) (rs1800872 and rs1800896). The genotypic and allelic frequencies of the aforementioned polymorphisms in the RM women and controls have been presented in Table 6. The distribution of genotypes and alleles did not differ significantly amongst cases and controls for SNP rs1800872 (P = 0.078: 0.23, respectively). However, a higher percentage of heterozygous genotype (CA) was reported to be present in controls (36.9% vs. 43.7%). Further, no association towards disease risk was found under all the studied genetic models (dominant, recessive and co-dominant). The genotypic distribution for rs1800896 has been observed to be statistically significant (P = 0.007) between both groups. The frequency of heterozygous genotype AG was noted to be significantly higher in cases than in the control group (49.7% vs. 39.5%). The differences in allele frequencies have also been found to be statistically significant (P = 0.003). The mutant genotype GG and heterozygous AG conferred a 1.7-fold and 2-fold risk, respectively towards the development of RM in women when compared with AA between cases and controls, (AA vs. AG; crude OR: 1.779; 95% confidence interval [CI]: 1.39–3.74; P = 0.006; AA vs. GG, crude OR: 2.139; 95% CI: 1.21–4.082; P = 0.02). The association of minor allele G with the susceptibility of RM is found statistically significant when compared with major allele A. Both the dominant and co-dominant models were found to provide 2-fold and 1.5-fold increased risk of RM, respectively after adjustment of age at menarche and BMI.

Haplotype analysis

The distribution of IL-10 (rs1800872 and rs1800896) haplotypes in women with RM and control women and total individuals is given in Table 7. Both the polymorphisms were neither found to be linked together

in the case nor in controls (D': 0.04; LOD: 0.02; $r^2 = 0.0$; D': 0.091; LOD: 0.08; $r^2 = 0.002$) [Figure 1]. Also, none of the haplotype combinations were conferring any significant risk or protection toward RM.

Discussion

During pregnancy, maternal immunity is suppressed by decidual cells and pregnancy depends on the induction of maternal tolerance to foetal tissues. During implantation, natural-killer cells migrate to the uterus and regulate the release of cytokines that promote or inhibit trophoblast invasion.[18] The allogeneic foetus will be protected against cytotoxic T-lymphocytes infiltration by an increase in Th2 cells and a decrease in Th1 cells. The secretions of Th1 cells and macrophages are decreased by IL-10, which is generated by cytotrophoblasts and decidual T cells to protect the foetal-placental interface.[19] IL-10 promoter polymorphisms −1082A>G, −819C>T and −592C>A have been reported to contribute to dysregulated IL-10 production which can lead to miscarriage.

The present age-matched case–control study examined promoter region polymorphisms of IL-10 in the region of Punjab in India. In this study, a significant difference in age at menarche amongst RM women and control women has been noticed which has also been reported

Table 5: Distribution of females according to the age and number of miscarriages

Age (years)	Number of miscarriage								
	2M	3M	4M	5M	6M	7M	8M	10M	Total, *n* (%)
20–24	8	2	-	-	-	-	-	-	10 (0.5)
25–29	54	34	7	5	2	-	-	-	102 (51.2)
30–34	36	23	3	2	1	-	-	-	65 (32.5)
35–39	7	7	1	-	2	1	2	1	21 (10.5)
40–45	-	1	-	-	-	-	-	-	1 (0.5)
Total	105	66	12	7	5	1	2	1	199

M=Miscarriages

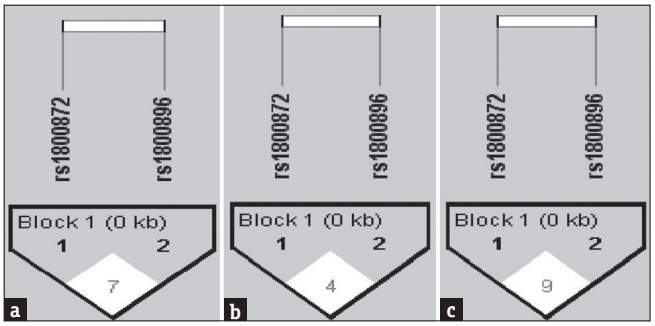

Figure 1: Linkage disequilibrium plot for interleukin-10 polymorphisms, (a) total cases, (b) total controls, (c) total studied samples (D′ <1, $r^2 = 0$ and LOD < 2)

Table 6: Genotype and allele frequency of interleukin-10 polymorphisms (rs1800872 and rs1800896) between cases and controls

SNP	Genotypes/alleles	Cases, *n* (%)	Controls, *n* (%)	*P*	OR (95% CI)	*P*	AOR	*P*
rs1800872	CC	104 (52.2)	96 (44.6)	0.07	Reference	-	-	
	CA	73 (36.6)	94 (43.7)		0.71 (0.47–1.08)	0.11	0.72 (0.47–1.09)	0.12
	AA	22 (11.1)	25 (11.6)		0.81 (0.43–1.53)	0.52	0.94 (0.50–1.75)	0.84
	C	281 (70.6)	286 (66.5)	0.23	Reference			
	A	117 (29.3)	144 (33.4)		0.82 (0.62–1.11)	0.20		
Dominant model	CA+AA	95 (47.7)	119 (55.3)		0.74 (0.50–1.09)	0.12	0.75 (0.50–1.11)	0.15
	CC	104 (52.2)	96 (44.6)					
Recessive Model	AA	22 (11.1)	25 (11.6)		0.94 (0.51–1.74)	0.85	0.99 (0.53–1.85)	0.9
	GG+GA	177 (88.9)	190 (88.3)					
Co-dominant model	AA	22 (11.05)	25 (11.63)		0.84 (0.61–1.11)	0.219	0.74 (0.49–1.10)	0.14
	CA	73 (36.68)	94 (43.72)					
	CC	104 (52.27)	96 (44.65)					
rs1800896	AA	72 (36.18)	110 (51.16)	0.007*	Reference			
	AG	99 (49.75)	85 (39.54)		1.77 (1.17–2.69)	0.006*	1.96 (1.27–3.02)	0.002*
	GG	28 (14.07)	20 (9.30)		2.13 (1.12–4.08)	0.02*	2.28 (1.16–4.46)	0.016*
	A	243 (61.05)	305 (70.93)	0.003*	Reference			
	G	155 (38.9)	125 (29.07)		1.55 (1.16–2.07)	0.002*		
Dominant model	AG+GG	127 (63.82)	105 (48.84)		1.85 (1.25–2.74)	0.002*	2.01 (1.34–3.03)	0.001*
	AA	72 (36.18)	110 (51.16)					
Recessive model	GG	28 (14.07)	20 (9.30)		1.6 (0.87–2.94)	0.13	1.73 (0.93–3.24)	0.08
	AA+AG	171 (85.93)	195 (90.70)					
Co-dominant model	GG	28 (14.07)	20 (9.30)		1.56 (1.16–2.09)	0.003*	1.572 (1.056–2.339)	0.026*
	AG	99 (49.7)	85 (39.54)					
	AA	72 (36.18)	110 (51.16)					

*P<0.05. OR=Odds ratio, CI=Confidence interval, AOR=Adjusted OR

Table 7: Distribution of haplotype observed for the two polymorphisms of the interleukin-10 amongst the cases and controls

Haplotypes	Cases (*n*=199)	Controls (*n*=215)	OR (CI 95%)	*P*
CA	170 (0.426)	199 (0.463)	0.87 (0.66–1.14)	0.303
AA	111 (0.280)	106 (0.246)	1.18 (0.87–1.61)	0.290
CG	73 (0.184)	87 (0.202)	0.88 (0.62–1.24)	0.471
AG	44 (0.110)	38 (0.088)	1.28 (0.81–2.03)	0.287

*Level of significance $P<0.05$. Order of SNPs in IL-10 haplotypes: rs1800872, rs1800896. OR=Odds ratio, CI=Confidence interval, IL-10=Interleukin-10, SNPs=Single-nucleotide polymorphisms

by many investigators.[20-25] However, few authors did not report any significant difference concerning the age at menarche between cases and controls.[26,27] The underlying mechanisms acting for associating early menarche and RM are still biologically conceivable. Increased oestradiol level in adulthood has been reported in women with early menarche age.[28,29] Further, an increased level of oestradiol on the 3rd day of the menstrual cycle is indicative of poor ovarian reserve in women.[30-33]

Distribution of RM women with respect to the number of miscarriages and age was done which showed that a maximum number of women (51.12%) belonged to the age group of 25–29 years, around 11% of females were of advanced age in the study [Table 5]. The rate of miscarriage is as low as 5% in young women, according to the study given by Regan *et al.*[34] Poland *et al.* suggested that the risk of miscarriage increases with the number of previous miscarriages.[35] In the present study, it was revealed that with increasing age number of miscarriages also increases [Table 4]. Tabcharoen *et al.* also suggested that a decline in the fertility rate is strongly associated with advancing age, preferably after the mid-30s, and women who conceive at this age are at higher risk of pregnancy complications.[36]

In the current study, it was observed that the frequency of primary RM was more than secondary miscarriages (91.93% vs. 8.07%), which follows previous studies that reported a higher number of females with primary RM than secondary RM.[37,38] However, in a study from Israel, a higher frequency of women with secondary RM was recorded, the authors postulated this increased incidence may be due to high concern regarding fertility-related problems.[39] In the present study, most of the females experienced early pregnancy loss (56.2%), followed by early late (32.5%) and late (11%) [Table 5]. Similarly, the percentage of late miscarriages (22.60%) has been reported by Zammiti *et al.*,[40] but they noticed a higher percentage of early late loss (57.10%) in their study as compared to the present study.

All the females with RM in the present study reported no intake of alcohol [Table 3]. Whereas, in the control group only a single woman reported alcohol intake belonged to an urban area. The National Family Health Survey (NFHS)-4 of India documented that 0% of females reported to alcohol intake in the rural region of India and 0.1% of females in the urban region of India were taking alcohol. The present study is consistent with NFHS-4. In other studies, alcohol consumption in females has been significantly associated with the risk of spontaneous miscarriages and RM.[41,42]

BMI has been reported extensively to be associated with the increased risk of spontaneous abortions, gestational diabetes, pre-eclampsia, RM, endometriosis and polycystic ovary syndrome.[43-48] In the present study, BMI was not associated with the risk of RM as there was no difference amongst the BMI of cases and controls [$P = 0.691$; Table 3] and was in agreement with the results of Toft *et al.* (2012), Ispasoiu *et al.*, Liu *et al.*, Halim and Lubis, Romero *et al.* and Wani *et al.*[49-54] The present findings contradicted to the results of Zammiti *et al.*, Costa *et al.*, Alkhuriji *et al.* and Shen *et al.*[40,55-57]

The regulation of cytokine secretion in the maternal-foetal interface plays a pivotal role in the process of trophoblast invasion and placentation. The IL-10 polymorphisms (rs1800872 and rs1800896) were investigated in RM cases and controls. In the current study, the risk association of rs1800872 polymorphism could not be established as the genotypic and allelic frequency amongst cases and controls did not differ significantly [Table 6]. Our study is in line with the study performed on Indian, Greek; Argentines and Saudi Arab women.[56,58-60] However, a study by Zammiti *et al.* on Tunisian women and by Zhang *et al.* on Chinese RM women reported significant association and risk towards RM.[61,62]

Genetic modification in cytokine production genes partially controls an individual's immunological response. Early immunologic alteration detection might avoid poor pregnancy outcomes. The −1082A/G (rs1800896) polymorphism of the IL-10 gene's promoter region has been extensively studied due to its reported involvement in the abnormal expression of IL-10 in RM.[21,61,63-68] It has also been revealed by a study conducted on a hypertensive rat model that IL-10 contributes to the normalisation of blood pressure and endothelial function. These results highlight the significance of this cytokine in a healthy pregnancy.[58] Our study demonstrated statistically significant differences in genotypic and allelic frequency of rs1800896 polymorphism between RM cases and

controls (P = 0.007 and P = 0.003 respectively). The heterozygous genotype mutant (GG) genotype, and mutant allele (G) confer 1.77-fold, 2.1-fold and 1.55-fold, respectively risk towards the progression of RM [Table 6]. In addition, dominant (P = 0.002) and co-dominant (P = 0.003) models exhibited a significant association with RM. A case–control study carried out in the Indian subcontinent has indicated a significant association between IL-10 (rs1800896) polymorphism and RM under the dominant (P = 0.002) and additive models (P = 0.01), and also confirmed that genetic models provide 2.6 and 1.98 fold risk for RM, which is consistent with the findings of our study.[58] In addition, our results were also supported by the findings of several studies.[64-66] A meta-analysis by Peng *et al.* found a significant association between −1082A/G polymorphism and RM risk, and they also concluded that G allele, as well as the GG genotype, increased the risk.[69] A recent meta-analysis by Gu *et al*. also found a significant association of IL-10 −1082A>G polymorphism with respect to RM in Asians.[63] However, studies by Kamali-Sarvestani *et al.*; Bohiltea and Radoi; Zammiti *et al.* and Qaddourah *et al.* have reported non-significant findings.[21,61,67,68] The observed disparities provide clear evidence that polymorphisms have a population-specific effect on the likelihood of having RM. A comparison of the frequency distribution of various studies worldwide in the context of RM and promoter region variants has been represented in Tables 8 and 9.

Haplotype distribution of IL-10 polymorphisms did not reveal the association of any haplotype with disease outcome [Table 7]. Also, none of the genotype combinations of *IL-10* polymorphisms (−592 and −1082) was associated with disease risk in the present study. Under two loci model of IL-10 IL-10 −592C>A and −1082A>G polymorphisms, Zammiti *et al.* also did not find an association for patients (RM women) but a weak LD was seen in controls for the −592C and −1082G alleles (D′ =0.049; P = 0.056) in their study.[61] Similarly, Qaddourah *et al*. did not report any strong LD amongst rs1800872 and rs1800896 haplotypes with IL-10 promoter region polymorphisms.[21]

Our study had several strengths which include the sufficient power of the study and homogenous sampling (only from the Punjabi population). Limitations of present study include only two IL-10 polymorphisms were examined, and hospital-based sampling limited the generalisation of the findings to the entire population.

Conclusion

The present study is the first of its kind to examine the association of promoter region polymorphisms of IL-10 with the RM women in India. Our study revealed an association of IL-10 −1082A > G variant with RM but no association of *IL-10* rs1800872 polymorphism was seen with RM. However, further research is needed to determine how these polymorphisms affect a woman's propensity for RM. In addition, evaluation of IL-10 expression is needed in relation to RM and maintaining successful pregnancies.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data used in the present study are available by the corresponding author on request.

Table 8: Comparison of genotype and allele distribution of IL-10 rs1800872 polymorphism in the present study with international and Indian studies

Studies	Country (Ethnicity)	Cases			Controls			*P*
		Genotypes (%)			Genotypes (%)			
		CC	CA	AA	CC	CA	AA	
Present study	Indian (South Asian)	104(52.2)	73(36.6)	22(11.05)	96(44.6)	94(43.72)	25(11.63)	0.07
Alkhurji *et al*.[55]	Saudi Arabia (Asia)	28(43.1)	28(43.1)	9(13.8)	32(49.2)	23(35.4)	10(15.4)	0.53
Bahadori *et al*.[72]	Iran (Asian)	8(9.4)	34(40)	43(50.6)	22(21.2)	49(47.1)	33(31.7)	0.01*
Bohiltea and Radoi,[68]	Romania (Caucasian)	30(69)	33(69)	6(69)	35(64)	26(64)	3(64)	0.36
Cochery-Nouvellon *et al*.[73]	Italy (Caucasian)	24(40)	34(56.7)	2(3.3)	65(57.0)	37(32.5)	12(10.5)	0.005*
Maskhina *et al*.[74]	Russia	123(43.31)	136(47.89)	25(8.80)	155(54.58)	116(40.85)	13(4.58)	0.01*
Liu *et al*.[50]	China (East Asian)	91(45.5)	79(39.5)	30(15.0)	148(49.3)	116(38.6)	36(12.0)	0.5
Parveen *et al*.[57]	Indian (South Asian)	73(57)	41(32)	14(11)	77(11)	66(41)	18(11)	0.25
Zammiti *et al*.[60]	Tunisia (African)	206(58.9)	93(26.6)	51(14.1)	134(67)	41(20.5)	25(12.5)	0.15

*P<0.05 considered significant

Table 9: Comparison of genotype and allele distribution of IL-10 rs1800896 polymorphism in the present study with international and Indian studies

Authors	Country (Ethnicity)	Cases			Controls			*P*
		Genotypes (%)			Genotypes (%)			
		AA	AG	GG	AA	AG	GG	
Present study	India (Asian)	72(36.18)	99(49.75)	28(14.07)	110 (51.16)	85 (39.54)	20 (9.30)	0.007*
Babbage *et al*.[75]	U.K (Caucasian)	8(19)	23(53)	12(28)	20(27)	41(56)	12(17)	0.26
Bahadori *et al*.[72]	Iran (Asian)	35(41.2)	33 (38.8)	17(20)	(40.4)	(40.4)	(19.2)	0.97
Bohiltea and Radio,[68]	Romania (Caucasian)	7(13.2)	28(52.8)	18(33.9)	11(17.18)	24(37.5)	29(45.3)	0.25
Liu *et al*.[50]	China (Asian)	38(13.38)	150(52.82)	96(33.8)	49(17.25)	107(37.68)	128(45.08)	0.001*
Daher *et al*.[76]	Brazil (Caucasian)	13(30)	19(44)	11(26)	45(44)	43(41)	16(15)	0.21
Zammiti *et al*.[60]	Tunisia/ Bahrain (African)	87(26.6)	185(52.9)	72(20.6)	54(27.0)	107(53.5)	200(19.5)	<0.001*
Parveen *et al*.[57]	India (Asian)	86 (43.0)	99 (49.5)	15 (7.5)	180(60.0)	108 (36.0)	12(4.0)	0.007*
Ma *et al*.[12]	China (Asian)	683 (88.1)	88 (11.4)	4 (0.5)	685 (85.1)	113 (14.0)	7 (0.9)	0.18
Kamali-Sarvestani *et al*.[67]	Iran (Asian)	50 (41)	49 (40)	23 (19)	81 (51)	59 (37)	19 (12)	0.14
Karkhukopri *et al*.[77]	Finland (Finish)	13 (34.2)	16 (42.1)	9 (23.7)	44 (33.6)	64 (48.9)	23 (17.5)	0.64

*P<0.05 considered significant

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