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Research article

Interleukin-10 Gene Promoter Polymorphisms in Women with Recurrent Spontaneous Abortion in Gaza Strip-Palestine

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ABSTRACT

Background: This study was conducted in order to investigate the relationship between interleukin-10 promoter variants -1082A/G, -819C/T, and -592C/A and recurrent spontaneous abortion (RSA) in Palestinian women residing in Gaza strip.
Methods: Retrospective case control study was performed between September 2013 and March 2014. Two hundred Palestinian women with RSA and 200 control women without previous history of RSA were included in the study. PCR allele-specific amplification (PCR-ASA) method was used for IL-10 genotyping.
Results: None of the investigated IL-10 promoter gene polymorphisms showed significant association with increased risk of RSA. Interestingly, the high observed/expected heterozygosity ratio in the IL-10 -1082A/G points to a "heterozygote advantage" of this particular genotype.
Conclusion: The examined IL-10 promoter gene polymorphisms are not associated with increased risk of RSA in Gaza strip-Palestine. Examining additional IL-10 variants and other genes' polymorphisms involved in Th1-Th2 immune balance in RSA cases is therefore recommended.

Keywords: RSA, Interleukin-10, Gene Polymorphism, Gaza Strip, Palestine.

INTRODUCTION

Recurrent spontaneous abortion (RSA), defined as three or more consecutive pregnancy losses before the 20th week of gestation, is a distressing problem particularly to Palestinian families. About 1-3% of women experience RSA[1].
Known etiologic factors of RSA include autoimmune disorders (~20%), endocrine factors (17-20%), anatomic abnormalities (10-15%), genetic abnormalities (2-5%) and infectious factors (0.5-5%). However, the causes of more than 50% of RSA remain idiopathic[2].

Recently, dysregulated immunity was proposed as a potential mechanism underlying RSA[3,4]. Unbalanced production of certain cytokines may play a role in unexplained RSA and also some cytokine gene polymorphisms may affect the level of cytokine production. For instance, IFN-γ and TNF-α inhibit trophoblast growth and differentiation, whereas IL-4, IL-10 and IL-13 may promote embryo development and placentation. On the basis of these observations, a Th2-type dominant response has been associated with normal pregnancy, whereas a Th1-type response has been related to pregnancy failure[5,6].

Among Th2 cytokines, IL-10 plays a key role in Th2 immunity. *IL-10* gene is located on human chromosome 1 (1q31–q32) and many single-nucleotide polymorphisms (SNPs) were reported in the proximal (at position -1082A/G, -819C/T and -592C/A) and distal regions of this gene and were reportedly involved in *IL-10* transcription rate, thereby directly affecting its production level[7,8].

Reduced expression of *IL-10* is implicated in RSA, due to defective maternal immune tolerance (causing early miscarriages) or placental vascular insufficiency (causing late losses). IL-10 production is in part inherited, and IL-10 gene variants associated with reduced IL-10 expression have been analyzed for their association with RSA[9].

The production of IL-10 varies according to specific alleles at position –1082, –819, or –592[6]. The association of *IL-10* promoter SNPs with recurrent miscarriage has been reported by several studies but with inconclusive findings[10-13].

The present study was designed in order to investigate the association between *IL-10* gene polymorphisms and RSA among Palestinian women with RSA residing in Gaza Strip.

METHODS

Study population

The study was conducted on 200 Palestinian women, 18–35 years old, who had at least two RSAs ≤20 weeks of gestation. Age and ethnicity matched 200 women with at least two live births and without a previous history of abortion or pregnancy-associated complications served as the control group. Informed consent was obtained from all participants, and approval for conducting the study was obtained from the local ethics committee.

DNA extraction and polymorphism determination

Genomic DNA was extracted from EDTA-blood samples using Wizard Genomic DNA purification Kit (Promega, USA) following the manufacturer's instructions.

IL-10 genotyping was performed using PCR allele-specific amplification (PCR-ASA) as described by other investigators [14-18], using the primers shown in Table 1.

Positive controls were selected by amplifying and sequencing two regions of *IL10* promoter; the first containing -592 and -819 SNPs (Control 1) for -592C/A and -819C/T, while the second contained the -1082 SNP (Control 2).

Table 1: Primers and lengths of PCR products

SNP	Primer	Primer Sequence (5' to 3')	Size (bp)
-592C/A	Common Reverse	GCTCACTATAAAAATAGAGACGG	223
	Forward C	CTGGCTTCCTACAGG	
	Forward A	GACTGGCTTCCTACAGT	
-819C/T	Common Reverse	AGGATGTGTTCCAGGCTCCT	233
	Forward C	CCCTTGTACAGGTGATGTAAC	
	Forward T	ACCCTTGTACAGGTGATGTAAT	

-1082A/G	Common Reverse	GTAAGCTTCTGTGGCTGGAGTC	161
	Forward A	AACACTACTAAGGCTTCTTTGGGT A	
	Forward G	AACACTACTAAGGCTTCTTTGGGTG	
Control 1	Forward	AATCCAGACAACACTACTAAGG	500
	Reverse	TTCCATTTTACTTTCCAGAG	
Control 2	Forward	TTTCCAGATATCTGAAGAAGTCCTG	313
	Reverse	GTAAGCTTCTGTGGCTGGAGTC	

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) equation was used to calculate the expected genotypes frequencies. Difference between expected and observed genotypes was assessed by Chi square (X^2) test. P-values less than 0.05 were considered statistically significant. The frequencies of the alleles and genotypes were compared between patient and control groups by X^2 test. The odds ratio (OR) and 95% confidence interval (CI) were also estimated in order to test the relation between RSA and the investigated polymorphisms.

RESULTS

-592 C/A *IL-10* Gene Polymorphism and RSA

Genotype and allele frequencies of *-592 C/A IL-10* gene polymorphism were not significantly different between RSA patients and controls (Table 2). Moreover, statistical analyses of the genotypes under recessive, dominant, additive and co-dominant models (data no shown) indicated no significant difference between the two study groups.

-1082 A/G *IL-10* Gene Polymorphism and RSA

Regarding *IL-10* -1082A/G polymorphism, the significantly higher homozygous (GG and AA) genotypes in the controls (Table 3) and the lack of significant difference in terms of the frequency of A or G alleles between RSA and control groups (Table 3) imply that neither allele constitutes a risk factor for RSA in the study sample.

-819 C/T *IL-10* Gene Polymorphism and RSA

Genotype and allele frequencies of *-819 C/T IL-10* gene polymorphism were not significantly different between RSA patients and controls (Table 4). Moreover, statistical analyses of the genotypes under recessive, dominant, additive and co-dominant models (data no shown) revealed no significant difference between the two study groups.

Table 2: Frequency of the -592 C/A *IL-10* gene polymorphism among RSA patients and control subjects

Polymorphism	Genotype/Allele	Patient N= 200	Controls N=200	Odds Ratio (95% CI)	P-value
-592 C/A	**CC**	74 (37.0%)	92 (46.0%)	0.69 (0.46 to 1.03)	**0.07**
	CA	113(56.5%)	95 (47.5%)	1.44 (0.97 to 2.13)	**0.07**
	AA	13 (6.5%)	13 (6.5%)	1.00 (0.45 to 2.21)	**1.00**
	C allele **A allele**	261 (65.25%) 139 (34.75%)	279 (69.75%) 121 (30.25%)	0.81 (0.61 to 1.10)	**0.17**

Table 3: Frequency of the -1082 A/G *IL-10* gene polymorphism among RSA patients and control subjects

Polymorphism	Genotype/Allele	Patient N= 200	Controls N=200	Odds Ratio (95% CI)	P-value
-1082 A/G	AA	46 (23.0%)	64 (32.0%)	0.63 (0.41 to 0.99)	**0.04**
	AG	150 (75.0%)	120 (60.0%)	2.00 (1.30 to 3.07)	**0.001**
	GG	4 (2.0%)	16 (8.0%)	0.23 (0.08 to 0.72)	**0.01**
	A allele G allele	242 (60.5%) 158 (39.5%)	248 (62.0%) 152 (38.0%)	0.94 (0.71 to 1.25)	**0.66**

Table 4: Frequency of the -819 C/T *IL-10* gene polymorphism among RSA patients and control subjects

Polymorphism	Genotype/Allele	Patient N= 200	Controls N=200	Odds Ratio (95% CI)	P-value
-819 C/T	CC	123 (61.5%)	111 (55.5%)	1.28 (0.86 to 1.91)	**0.22**
	CT	68 (34.0%)	73 (36.5%)	0.90 (0.59 to 1.35)	**0.60**
	TT	9 (4.5%)	16 (8.0%)	0.54 (0.23 to 1.26)	**0.15**
	C allele T allele	314 (78.5%) 86 (21.5%)	295 (73.75%) 105 (26.25%)	1.30 (0.94 to 1.80)	**0.12**

Hardy-Weinberg equilibrium

Analysis of the observed and the calculated expected genotype frequencies of -592 C/A and -819 C/T polymorphisms in the control group showed that the distribution of both genes' genotypes are in Hardy-Weinberg equilibrium. The observed genotypic distribution of -1082A/G polymorphism, however, deviated significantly from HWE expectation. The high observed/expected heterozygosity ratio of points to a "heterozygote advantage" in this particular this SNP.

DISCUSSION

Successful pregnancy depends on maintaining a fine balance between Th1 and Th2 immunity[19]. This was reportedly characterized by a shift to Th2 (humoral) immunity, which involves augmentation in the production of Th2 cytokines e.g., IL-10/ IL-4/IL-5/L-13 accompanied by attenuation of Th1 cytokines e.g., IFN-γ synthesis[20,21].

Cytokine production is mainly controlled at the gene transcription level and due to variation in genetic background and polymorphism - SNPs in particular - in cytokine genes, it is conceivable that individuals differ in their cytokine levels. For instance, *IL-10* gene 5´-flanking region, which controls transcription, is polymorphic with two microsatellites and three SNPs "-1082 A/G, -819 C/T, and -592 C/A"[22].

Various studies from different populations have been carried out to assess the association between *IL-10* gene polymorphisms and RSA, either at the genotypic or the haplotypic level. Three promoter region SNPs (-1082 A/G, -819 C/T, and -592 C/A) have been implicated as potential risk factors for RSA.

However, conflicting results have been reported on the association of IL-10 promoter variants with RSA. For rs1800896 (–1082A/G), no significant association with RSA was found in this study and this is in agreement with results from the UK[23], Finnish[14], Brazilian[5], Argentinian[24], Iranian[10], Tunisian[15] and Bahranian[9] studies. In the contrary, our results are in disagreement with a study from North India, which documented strong association of rs1800896 (P=0.0004) with RSA[13]. Interestingly, the high observed/expected heterozygosity ratio points to a "heterozygote advantage" of this particular genotype. This heterozygote advantage could be related to as yet unidentified pathogen infection in Gaza strip population and deserves further investigations. Indeed,

Hoebee et al. (2004) have reported "heterozygote advantage" at the IL-10 (-592 C/A) for respiratory syncytial virus (RSV) infections[25].

Based on the genotype and allele frequency results we can conclude that the *IL-10* -592 C/A polymorphism does not represent a risk factor for RSA in our population. Lack of association between RSA and this IL-10 gene polymorphism is in agreement with the findings reported by (Babbage et al., 2001; Prigoshin et al., 2004 and Alkhuriji et al., 2013)[23,24,26] but contradicts those of (Kamali-Sarvestani et al., 2005 and Zammiti et al., 2006)[10,15] who demonstrated an association between IL-10 -592C/A promoter polymorphism and RSA.

Lack of association between RSA and *IL-10* gene -819 C/T promoter polymorphism observed in this study is congruent with the findings reported by other investigators[10,14,23,27], but contradicts the findings of Parveen et al. (2013) and Zammiti et al. (2006)[13,15].

Discrepancy between results of genetic association studies like those encountered here could be due to many reasons including population genetic variation (background) unrelated to the investigated alleles, presence of nucleotide polymorphism somewhere else in the examined gene e.g., in the coding or intronic regions, epigenetic alterations and linkage disequilibrium to other sequence variants in the vicinity of the studied loci.

Results of the current study imply that the investigated *IL-10* gene SNPs do not contribute to the risk of RSA in our population. Screening for additional IL-10 variants and other genes' polymorphisms involved in Th1-Th2 balance in RSA cases is recommended.

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