

The Role of IL-10 Gene in Abortion Incidence at the Last Pregnancy Trimester in Iraqi Women

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ABSTRACT

Background: The term "abortion" refers to the removal or evacuation of an embryo or fetus to end a pregnancy. As previously mentioned, there is a relation between the IL-10 gene and abortion.

Material and method: Genomic DNA was isolated from 100 samples of whole blood obtained from the vein included (50 individuals with mischarged women and 50 control individuals) with biological data collected from participants in this study. An allele-specific PCR technique used to amplify A>G (rs1800896).

Results: The findings of the current study shown in Table 3 showed a clear significant difference in the parameter levels in the abortion study group. Based on the genotype frequency, the serum levels of IL-10 AA, AG, and GG increased significantly in the abortion group compared with the control (9.635.3 versus 2.0981.02 p-value 0.001), (15.363.64 versus 2.150.53 p-value 0.001), and (8.615.24 versus 2.190.72 p-value 0.002). The findings of blood pressure related to rs1800896 AA, AG, and GG genotypes in systolic blood pressure were (132.661.99 versus 1200 p-value 0.001), (133.071.34 versus 1200 p-value 0.0001), and (132.341.86 versus 1200 p-value 0.001), while in diastolic blood pressure were (90.951 versus 800, P-value 0.005), (91.341.52 versus 800 p-value 0.002), and (91.021.11 versus 800 p-value =0.001) compared with control.

Conclusion: IL-10 rs181206 genetic polymorphism is associated with an increased risk of third trimester miscarriage, and also observed significant differences in IL-10 levels among different genotypes.

KEYWORDS: Abortion, Intrlokin 10, Polymorphism, SNP, Third trimester, Sample, Control

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INTRODUCTION

Recurrent spontaneous abortion (RSA), defined as three or more sequential abortions before the twentieth week of gestation, affects approximately one in 300. Precipitating elements include genetic, endocrine, anatomic, immunologic, and microbiologic factors; however, in almost 50% of the cases the etiology remains unexplained [1]. The unmodified word abortion generally refers to an induced abortion. The reasons why women have abortions are diverse and vary across the world. Reasons include maternal health an inability to afford a child, domestic violence, lack of support, feeling they are too young, wishing to complete education or advance a career, and not being able or willing to raise a child conceived as a result of rape or incest. Interleukin 10 (IL-10) is a cytokine with potent anti-inflammatory properties that plays a central role in limiting the host's immune response to pathogens, thereby preventing damage to the host and

maintaining normal tissue homeostasis [2]. Deregulation of IL-10 is associated with enhanced immunopathology in response to infection as well as increased risk for the development of many autoimmune diseases interleukin-10 was first reported by [3]. Under the name of cytokine synthesis inhibitory factor (CSIF) as a protein with the ability to inhibit the activity of inflammatory T-helper 1 (Th1)-type1 cells. Investigation led to the deduction that CSIF was the major factor that defined a difference between Th1-type and Th2-type T-cells as CSIF skewed T-cell activity toward inhibition [4]. Although originally defined as a product of Th2 cells, this cytokine has now been shown to be produced by a wide set of cell types, including both immune and non-immune cells. Reports also demonstrated that one mode of IL-10 regulation is through a feedback loop that curtails excessive inflammatory events [5]. For example, when monocytes are activated with lipopolysaccharide (LPS), a

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dual cytokine response is induced where pro-inflammatory cytokine production is countered by the production of IL-10, which began to flood the literature as a prominent cytokine that works in an autocrine and paracrine manner in response to the inflammatory limb of the immune system to sequester over-activation of proinflammatory signals [5].

MATERIALS AND METHODS

The total of 100 samples were taken from mid-October 2022 until to mid-October 2023. Collected from Women and Children's Hospital, also private medical laboratories in the Hilla province and Women Hospital in Dhi Qar province, divided into two groups: the first group is the control while the second group was the women who suffered from a miscarriage in the third trimester. Four mL of blood was drawn from patients and controls. Each sample was divided

into two parts, the first part 2 ml was placed in EDTA tube and used in molecular steps for DNA isolation and the second part was 2 ml used to isolate a serum and perform the biochemical tests. All samples were stored in a deep freezer at - 20 C° [6].

DNA Extraction

The DNA extraction kit provided by (Anatolia Inc., Turkey) used to isolate genomic DNA from 100 samples of whole blood collected from the vein puncture included (50 individuals with miscarried women and 50 control individuals).

Genotyping of IL10 polymorphisms

Allele Specific-PCR technique using 3 pairs of primer to amplify the 2 alleles of rs1800896 SNP, in a double PCR reaction. Primers Sequence listed in (Table1).

Table 1: The primer sequences used to amplify regions flanking of studied SNP.

Reference SNP ID	Primer Sequence	Product size	reference
IL-10 A>G (rs1800896)	Common primer: 5'-CAGCCCTTCCATTTACTTTC-3'	529bp	[7]
	G allele primer: 5'-TACTAAGGCTTCTTTGGGAG		
	A allele primer: 5'-CTACTAAGGCTTCTTTGGGAA-3'		

0.5 l of an isolated DNA solution, 4 l of nuclease-free water, 1 l of MgCl₂, 1.5 l of the outer primer, and 1.5 l of the inner primer were added to the tubes, for a total volume of 25 l, for each PCR process. For the PCR assay, the cycling conditions were 95°C for 5 minutes, then 35 cycles of touchdown reactions at 95°C for 30 seconds for the first cycle, continuing at 55°C for 30 seconds in the annealing step of the remaining cycles, and extension at 72° for 30 seconds with a final extension step at 72° for 10 minutes.

Cytokines (10) quantification

Serum levels of IL-10 were quantified by enzyme-linked immunosorbent assays (ELISA) using the predesigned kit as per the manufacturer's instructions (Elabscience Biotechnology Inc., USA) in all subjects enrolled for the present investigation.

Statistical Analysis

The statistical package for social science (SPSS) software, version 10 (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp. Chicago, USA) was used to analyze all of the data. Continuous variables were represented as the mean standard deviation (SD), whereas genotypes and the alleles were shown as frequencies and percentages. χ^2 , *p*-value, odd ratio, Confidence interval and Duncan multiple ranges tests were obtained [7].

RESULTS AND DISCUSSION

Table 2: Polymorphism and allele frequency of IL-10

Genotypes	Control N=50 , N(%)	3 rd Trimester N=50 , N(%)	Control versus 3 rd Trimester	
			OR (95% CI)	χ^2 / <i>p</i> -value
AA	30(60)	20(40)	0.48 (0.18 to 1.27)	1.46/0.143
AG	11(22)	15(30)	2.04 (0.78 to 5.35)	1.45/0.14
GG	9(18)	15(30)	2.5 (0.91 to 6.8)	1.79/0.04
Allele				
A	71(71)	55(55)	0.49 (0.27 to 0.89)	2/0.02
G	29(29)	45(45)	2 (1.11 to 3.59)	2.32/0.01

This table shows the interpretation of the results between patients and control. GG genotype frequency higher significantly with risk in comparison with control(30 versus

18% OR = 2.5; *p*-value 0.04).The studies related of rs1800896 polymorphism with IL-10 gene and its association with abortion were limited. The findings of this study related

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with A and G alleles frequency of rs1800896 is linked with a higher risk of pre-eclampsia during miscarriage. The results are consistent with previous research that found significant agreed with [5],[6].

Results in table 3 showed a clear difference of the parameter's levels in abortion study group. Serum level of IL-10 was increased significantly in GG genotype at abortion group comparing with control (8.61±5.24 versus 2.19±0.72; p=0.002). This result disagree [1]. This study found the levels

of Systolic Blood Pressure, Diastolic ,Blood Pressure were increased significantly in AA, AG, GG genotypes compared with control (132.66±1.99 versus 120±0 p- value 0.001), (133.07±1.34 versus 120±0 p-value 0.001), (132.34±1.86 versus 120±0 p- value 0.001), (90.95±1 veruse 80±0 p-value =0.002), (91.34±1.52 veruse 80±0 p-value=0.002), (91.02±1.11 versus 80±0 p-value 0.001). This result agree [7],[8],[9].

Table 3. Comparison serum level of IL-10 among the studied groups (Mean±SD)

Parameters	Groups	Genotypes frequency Mean±SD			p-value
		AA	AG	GG	
BMI	Control	27.15±2.06Aa	27.09±1.89Aa	25.27±2.01Aa	0.043
	Third	26.38±2.2Aa	26.79±2.025Aa	27.12±2.16222Ab	
	p-value	0.5	0.4	0.2	
Systolic Blood Pressure	Control	120±0B	120±0B	120±0B	0.53
	Third	132.66±1.99Aa	133.07±1.34Aa	132.34±1.86Aa	
	p-value	0.0001	0.0001	0.001	
Diastolic Blood Pressure	Control	80±0B	80±0B	80±0B	0.62
	Third	90.95±1A	91.34±1.52A	91.02±1.11A	
	p-value	0.0005	0.002	0.001	
IL-10	Control	2.098±1.02Ba	2.15±0.53B	2.19±0.72Ba	0.9
	Third	9.63±5.3Aa	15.36±3.64Aa	8.61±5.24Aa	
	P-Value	P<0.001	P<0.001	0.002	

CONCLUSION

In conclusion, this study suggests that the IL-10 rs181206 genetic polymorphism is associated with an increased risk of third trimester miscarriage, and also observed significant differences in IL-10 levels among different genotypes.

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Conflict of interests.

There are non-conflicts of interest.

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