

The Impact of IL-4 on Abortion Rates in Iraqi Women's Last Trimester Pregnancies

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ABSTRACT

The term "abortion" is used to describe the termination of a pregnancy through the surgical expulsion of a developing embryo or fetus. Whole samples were collected from Al-Zahraa Teaching Hospital in Wasit city From mid-October 2022 until mid-October 2023. The total number of samples was 100 divided into two groups: the first group is women who suffer from a miscarriage in the third trimester and the control group .Result: This table shows the interpretation of the results between patients and healthy people, where the homozygous CC genotype significantly decreased comparing with control (64 versus 86%, OR=0.18; p -value =0.03), while the CT heterozygous genotype was significantly high with risk in comparison with control (32 versus 8%; OR= 7.3; p -value 0.005). The study of polymorphism of the IL-4 gene and its association with abortion had limited available data. This study is the second to report on the association between polymorphism of IL-4 and abortion. showed a clear difference of the parameter's levels in abortion study group. Based on the mutant homozygous CC genotype. In the present study, the significant change in IL-4 concentration was observed due to the effect of the polymorphism in cases of abortion. These findings were consistent with a previous study that reported higher concentrations of IL-4 in women with abortion compared to normal women (P-value of 0.001). (Association between maternal circulating IL-4 levels and preeclampsia).

KEYWORDS: Abortion , IL-4 , Pregnancy , Trimester

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INTRODUCTION

The term "abortion" is used to describe the termination of a pregnancy through the surgical expulsion of a developing embryo or fetus [1]. Miscarriages, often known as "spontaneous abortions," account for between 30 and 40 percent of all abortions. [2,3]. To purposefully terminate a pregnancy, a procedure known as a "induced abortion" or, less commonly, a "induced miscarriage," is performed. The term "abortion" is commonly shorthand for "induced abortion" when not qualified. [4]. Immunity is an organism's resistance to pathogens. Immunity depends on both specialized and generalized factors. Nonspecific components protect against or eliminate many different diseases because of their broad antigenic specificity. Pathogen-specific immunity is acquired by some immune system cells through a process of adaptation with each new disease encountered [5].

Interleukins are principally responsible for the immune system's functioning, and rare deficits of some of them have been reported in cases of autoimmune disorders or immunological deficiencies. Monocytes, macrophages,

endothelial cells, and CD4 helper T lymphocytes are responsible for the majority of interleukins synthesis. They support the growth and differentiation of hematopoietic cells, including T and B lymphocytes.

Mice's development of spatial memory is also known to be influenced by interleukin receptors on astrocytes in the hippocampus [6]. IL-4; is secreted by antigen presenting cells and has been demonstrated to control inflammation during pregnancy in order to induce Th1 differentiation of CD4+ T cells while reducing proinflammatory cytokine production through STAT (Signal transducer and activator of transcription) transcription factors [4]. A subpopulation of T regulatory cells that limit intestinal pathology and improve host survival in *T. gondii* infection are also strengthened by IL-4 signaling [5]. Given that cytokines are subject to genetic controls, recent research suggests that cytokine genetic polymorphisms may function to control the levels of cytokine gene expression and their receptor [6]. Additionally, single nucleotide polymorphisms (SNPs) are diagnostic markers for conditions like leukemia, infertility, SARS-Cov-2 and only a few disorders have recently been

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mentioned linked with mutations in IL-4 [8–10]. The purpose of this study was to look for the role of IL-4 polymorphism in the incidence of spontaneous abortion in Iraqi women at last trimester of pregnancy.

MATERIAL AND METHOD

Sample collection

Whole samples were collected from Al-Zahraa Teaching Hospital in Wasit city From mid-October 2022 until mid-October 2023. The total number of samples was 100 divided into two groups: the first group is women who suffer from a miscarriage in the third trimester and the control group. Four mL of blood was drawn from patients and controls. Each sample was divided into two parts, the first part 2 ml, to isolate a serum and perform the biochemical tests, and the

second part 2 ml was placed in a tube with EDTA and used in molecular steps for DNA isolation. All samples are stored in a deep freezer at - 20 C°.

DNA Extraction

The isolated of genomic DNA from 100 samples of whole blood obtained from the vein included (50 individuals with mischaged women and 50 control individuals) using DNA extraction kit provided by (Anatolia Inc., Turkey)

Genotyping of IL-4 polymorphisms

Tetra-primer Amplification Refractory Mutation System (ARMS)- polymerase chain reaction (PCR) using 2 primer pairs to amplify the 2 alleles of SNP, respectively, in a single PCR reaction. primer Sequences of primer (5'→ 3') Sequences of primer (5'→ 3') primer (Table1).

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

Reference SNP ID	Primer sequence	Product size	Reference
IL-4 (C/T)	F: 5' CAGACAGTGGAGATGGAAGGAATG3'	247 bp	(Dehghanzadeh et al., 2016)
	R (C): 5' TCCCCAGCCCTCCCAGCG3'		
	R (T): 5' TCCCCAGCCCTCCCAGCA3'		

Each PCR reaction was performed in a total volume of 25µl, adding 12.5 µl of master mix, 0.5 µl of an isolated DNA solution, and nuclease-free water to the tubes 4 µl, 1 µl MgCl₂, along with 5 µl of outer primer, and adding 2 µl of inner primer. PCR cycling conditions for the assay were 95C° for 5 minutes, followed by 35 cycles of touchdown reactions at 95C° for 30 seconds for the first cycle, and then continuing at 55C° for 30 seconds in the annealing step of the remaining cycles with extension at 72C° for 30 second and a final extension step at 72C° for 10 minutes.

Cytokines (IL4) quantification

Serum levels of IL-4 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 27 (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.

RESULTS

Table 2: Polymorphism of IL-4

Genotypes	Control N=50 , N(%)	3rd Trimester N=50 , N(%)	Control versus 3rd Trimester	
			OR (95% CI)	χ^2/ p - value
CC	43(86)	32(64)	0.18(0.05 to 0.61)	2.77/ 0.003*
CT	4(8)	16(32)	7.3(0.1- 3.01)	2.9/0.005*
TT	3(6)	2(4)	0.91(0.14-5.8)	0.49/0.92
Allele				
C	90(90)	80	0.44 (0.19 to 1)	1.96/0.06
T	10(10)	20	2.25(1.08-5.86)	1.94/0.05

This table shows the interpretation of the results between patients and healthy people, where the homozygous CC genotype significantly decreased comparing with control (64 versus 86%, OR=0.18; *p*-value =0.03), while the CT heterozygous genotype was significantly high with risk in comparison with control (32 versus 8%; OR= 7.3; *p*-value 0.005). The study of polymorphism of the IL-4 gene and its association with abortion had limited available data. This

study is the second to report on the association between polymorphism of IL-4 and abortion.

Results in table 3 showed a clear difference of the parameter's levels in abortion study group. Based on the mutant homozygous CC genotype , BMI and serum level of IL-4 were decreased significantly in abortion group comparing with control (25.84±1.63 versus 28.09±1.69; *p*-value 0.04), (96.57±27.27 versus 118.31±16.3; *p*-value 0.001); while the systolic and the diastolic blood pressures were significantly

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high in abortion group comparing with control (132.76±1.68 versus 120±0; *p*-value 0.003), (91.4±1.38 versus 80±0; *p*-value 0.003). At the TC heterozygous genotype the levels of BMI, LH, Systolic and diastolic blood pressure were higher significantly in abortion group comparing with control (27.14±2.51 versus 25.4±2.02 *p*-value 0.1)(7.15±0.68 versus 5.58±5.12 *p*-value 0.0001)(132.97±1.96 versus 120, *p*-value 0.0002)(91.39±1.37 versus 80±0, *p*-value 0.0001). At the TT homozygous genotype the levels of LH, Systolic and diastolic blood pressure were higher significantly in abortion group comparing with control (26.82±1.88 versus 26.89±2.003 *p*-value 0.9)(9.25±8.91 versus 7.97±5.42 *p*-value 0.0001)(132.38±1.65 versus 120±0 *p*-value 0.0001)(90.62±0.73 versus 80±0 *p*-value 0.0003). The correlation between the location of the SNP in the coding region and the potential for this polymorphism to cause the disease was investigated in this study. A study analyzed the effect of the mutant and wild-type alleles on the expression levels of the IL-4 gene to modulate OF mRNA level

(Pathogenic implications for autoimmune mechanisms derived by comparative analysis of CD4+ versus CD8+ T cells). In addition to another study by Computational prediction models were used in a study to investigate evidence for a functional effect of rs181206. According to PolyPhen2, this variant is predicted to be "probably damaging," to protein as reported in the study. (A method and server for predicting damaging missense mutations)

The I-Mutant2.0 server was utilized to analyze the polymorphism and it was found that the change of amino acid from Leucine to Proline could decrease the stability of the protein as in table (4). The decrease in protein stability resulting from the amino acid change was attributed to a potential change in the helix to coil transition. The unique characteristics of proline, which lacks both free α -amino and free α -carboxyl groups, were also noted in the analysis. (I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure)

Table 3. Comparison serum level of IL-4 among the studied groups (mean±SD)

Parameters	Groups	CC	CT	TT	<i>p</i> -value
BMI	Control	26.89±2.003Aab	25.4±2.02Bb	28.09±1.69Ba	0.01
	Abortion	26.82±1.88Aa	27.14±2.51Aa	25.84±1.63Aa	0.2
	<i>p</i>-value	0.9	0.1	0.04	
LH	Control	7.97±5.42Aa	5.58±5.12Ba	6.43±5.54Aa	0.4
	Abortion	9.25±8.91Aa	7.15±0.68Aa	7.203±0.98Aa	0.4
	<i>p</i>-value	0.0001	0.0001	0.07	
Systolic Blood Pressure	Control	120±0B	120±0B	120±0B	--
	Abortion	132.38±1.65Aa	132.97±1.96Aa	132.76±1.68Aa	0.59
	<i>p</i>-value	0.0001	0.0002	0.003	
Diastolic Blood Pressure	Control	80±0Ba	80±0Ba	80±0Ba	--
	Abortion	90.62±0.73Aa	91.39±1.37Aa	91.4±1.38Aa	0.07
	<i>p</i>-value	0.0003	0.0001	0.003	
IL-4	Control	117.59±16.98Ba	121.61±16.9Ba	118.31±16.3Ba	0.7
	Abortion	139.48±31.51Aa	112.23±38.8Aa	96.57±27.27Aa	0.003
	<i>p</i>-value	P<0.001	0.001	P<0.001	

Significant difference at *P*<0.05

In the present study, the significant change in IL-4 concentration was observed due to the effect of the polymorphism in cases of abortion. These findings were consistent with a previous study that reported higher

concentrations of IL-4 in women with abortion compared to normal women (*P*-value of 0.001). (Association between maternal circulating IL-4 levels and preeclampsia).

Table (4) showed the mean±SD of IL-4 concentrations by ELISA technique in the cases group compared to the control group.

Biomarkers	Groups	Mean±SD	<i>P</i> -value
IL-4 ng/ml	Control	156.62±150	P<0.001*
	Third trimester	177±160.44	

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