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Study of IL-27 Polymorphisms with Chemical Factor in Abortion Women

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ABSTRACT

Disease-predictive single nucleotide polymorphisms (SNPs) are also found in cytokines, which are under the control of genetics and whose genetic variations operate to modulate the amounts of cytokine gene expression. Its objectives are. in women who have had abortions, limited polymorphism of il27 with free any pathogen. whole samples were taken from the Women's and Children's Hospital in the city of Hilla and the Women's Hospital in the city of Dhi-Qar The total number of samples was 200 divided into four groups: the first group is women who suffer from miscarriage in the first trimester, the second group is women who suffer from miscarriage in the second trimester, the third group who suffer from miscarriage in the third trimester, and the last group are women who are only pregnant. for detecting the level of IL-27 by ELISA while the allelic discrimination method was used for SNP IL-27. The results indicated the IL-27 serum concentration elevated with significant differences in recurrent abortion in both groups compared to healthy women, Also, recurrent abortion had significant differences compared to healthy women ($P<0.05$). Also, in recurrent abortion, the level of IL-27 for, GC, and CC genotypes showed significant differences compared to healthy and pregnant women ($P<0.05$). Also, in recurrent abortion, the level of IL-27In conclusion, the level of IL-27 in recurrent abortion women with free pathogen was higher than the recurrent abortion women, which may be due to the pro-inflammatory for l27. SNP of IL-27 has been represented as a risk factor in recurrent abortion women.

Keywords: *Polymorphisms, IL-27, Factor, Abortion Women*

INTRODUCTION

The term "abortion" refers to the removal or evacuation of an embryo or fetus to end a pregnancy [1]. Miscarriages, also known as "spontaneous abortions," are abortions that take place naturally and happen in 30% to 40% of pregnancies. [2,3]. An induced abortion, or less frequently "induced miscarriage," is when pregnancy is intentionally ended. Abortion, when used without modification, typically refers to an induced abortion. [4]. Immunity refers to an organism's capacity to fend off harmful microbes. Both specific and generic components contribute to immunity. Regardless of their antigenic makeup, the nonspecific components serve as barriers to or eliminators of a variety of infections. Some immune system cells can develop pathogen-specific immunity by adjusting to each new sickness they encounter [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-27 ; is secreted by antigen
presenting cells and has been demonstrated to co ntrol inflammation during pregnancy in order to induce Th1 differentiation of CD4+ T cells whil e reducing proinflammatory cytokine production through STAT (Signal transducer and activator of transcription) transcription factors [4].
A subpopulation of T regulatory cells that limit i ntestinal pathology and improve host survival in T. gondii infection are also strengthened by IL-27 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 differential therapeutic response. Asthma, colorectal cancer, rheumatoid arthritis, ovarian cancer, Crohn's disease, esophageal cancer, and nasopharyngeal carcinoma are only a few of the disorders that have recently been linked to IL-27 mutations [8–10]. The purpose of this study was to look at the serum levels of IL-27 and its polymorphism in women who had spontaneous abortions and a free pathogen.

MATERIAL AND METHOD

Sample collection

whole samples were taken from the Women's and Children's Hospital in the city of Hilla and the Women's Hospital in the city of Dhi Qar. Some of the medical laboratories such as the Al-Mustafa laboratory and the Al-Rawan laboratory in the city of Hilla. From mid-October 2022 until mid-October 2023.

The total number of samples was 200 divided into four groups: the first group is women who suffer from a miscarriage in the first trimester, the second group is women who suffer from a miscarriage in the second trimester, the third group who suffer from a miscarriage in the third trimester, and the last group are women who are only pregnant.

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 genomic DNA from 90 samples included (30 individuals with infertility women who suffer from obesity and 30 individuals with infertility women who suffer from T2DM and 30 healthy control individuals). The quality of DNA samples is also checked by electrophoresis on 0.8% agarose gel and was to be of a high integrity DNA with distinct bands at the top of the gel indicating high-quality nondegraded genomic DNA.

Genotyping of IL27 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

GACTTC AGCTTTACTCTTTGTCAAGACA Outer (F) GAATGAGCCGCACTT TTCCCC CTAGTTGTGTCTTCCG Oter(R) ATTGTGCAATGTGACGTCCTTTAGCTTG F inner(G|)

(C allele)TCAGACATCTCCAGTCCTA (R) inner

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.

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 categorical variables were shown as frequencies and percentages.

In order to compare parameter means between patients and controls, a oneway ANOVA test was utilized, followed by a Duncan multiple comparisons post hoc Chi-square test.

Each group's genotype and allele distributions were identified, and odds ratios (OR) and 95% confidence intervals (CI) for each were computed. In controls, the Hardy

Weinberg equilibrium was checked for deviation in the genotype distributions of SNPs. the p-value

RESULTS

Clinical characteristics of RSA and control groups. The case-control study included 150 RSA partitions that divide into three groups: first trimester 30 (22-34 years old).

TABLE 1: The clinical characteristics of cases and controls in the first-trimester group

Parameters	CONTROL, (50) n	FIRST, (50) n	P.value
Age Median (IQR)	29.5 (22-33)	30(22-34)	0.8
Diastolic Blood Pressure Median (IQR)	80	82 (81.4-83.2)	P<0.001
Systolic Blood Pressure Median (IQR)	120	122.4 (121.4-123.5	P<0.001
Hemoglobin, Mean±SD	13.29±1.41	10.06±0.55	P<0.001
TSH, (M±SD)	9.74±4.88	8.71±0.374	P<0.001
Progesterone, (M±SD)	24.64±25.71	6.105±0.64	P<0.001
AMH, (M±SD)	32.37±30.13	1.41±0.27	P<0.001

The distribution of selected epidemiologic and clinical factors in cases and controls such as diastolic Blood Pressure and Systolic Blood Pressure, Hemoglobin, TSH, and Progesterone. e similar between the cases and controls (all P<0.001,) shown in Table1

TABLE 2: The clinical characteristics of cases and controls in the second-trimester group

Parameters	CONTROL, (50) n	Second(50)	
Age Median (IQR)	29.5 (22-33)	30(22-33)	P=0.89
Systolic Blood Pressure Median (IQR)	120	127.3 (126.7-127.8	P<0.001
Diastolic Blood Pressure Median (IQR)	80	82.11 (81.2-83)	P=0.04
Hemoglobin, Mean±SD	13.29±1.41	9.23±.437	P<0.001
TSH, (M±SD)	9.74±4.88	10.56±0.33	P<0.001

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TABLE 3: The clinical characteristics of cases and controls in the third-trimester group

Parameters	CONTROL, (50) n	Third, (50) n	P.value
Age Median (IQR)	29.5 (22-33)	30(22-34)	P=0.8
Systolic Blood Pressure Median (IQR)	120	132.5 (131.3-133.7)	P<0.001
Diastolic Blood Pressure Median (IQR)	80	90.4 (90.1-91.9)	P<0.001
Number Of Miscarriages Median (IQR)	-	2 (1-2)	-
Hemoglobin, Mean±SD	13.29±1.41	8.61±0.451	P<0.001
TSH (M±SD)	9.74±4.88	13±0.55	P<0.001
Progesterone (M±SD)	24.64±25.71	2.93±0.59	P<0.001

Genetic analysis: This mutation will be distributed to three groups(first group P value= 0.04, second group P value =0.03*, third group P value =0.37)

Clear we significant notice between the first group and the healthy group,(P=0.04<<0.05) as well as a second group with healthy people(P=0.03.<005, but we do notice the significance with a third group(Pvalue =0.04>0.05) Contrary to this, the GGgenotype of IL27 was associated with a higher risk of presenting RSAwih first group (OR =0.39 CI =0.16-0.98, χ2/ P value=1.97/0.03*)and higher risk when compare the second group with control (OR=0.35 Cl=0.15-0.99, χ2/ P value 1.95/ 0.02*), as the prevalence of this genotype was detected to be significantly lower in RSAin third group cases than controls (OR =0.4 Cl=0.15-0.01χ2/ P value 1.92 /0.04*).When GC genotype of IL27(OR=2.5 (1.01-6.24) , χ2/ P value 1.99/0.04*in first group.higer risk with scond group (oR=2.51 Cl= 1-6.29) χ2/ P value 1.97/0.04*.

the associated with a higher risk of presenting RSAwih this group (OR =2.75 CI =0.18 -2.1), χ2/ P value=0.75/0.45*). Genotype cc with first group (OR =0.92 CI =0.29-2.27), χ2/ P value=0.14 /0.88) scond group (oOR=0.92 Cl(0.29-2.72) χ2/ P value 0.14/0.88)third group (OR=1.59 Cl=0.3-1.15 , χ2/ P value =0.89/0.37)

Groups	Frequency n (%)	G1 versus G2	G1 versus G3	G1 versus G4						
	G1 control N=50 N(%)	G2 1st trimister N=50	G3 2nd trimister N=50	G4 3rd trimister N=50	OR (95% CI)	χ2/ P value	OR (95% CI)	χ2/ P value	χ2/ P value	
Genotype										
TT	29(58)	23(46)	22(44)	20(40)	0.39 (0.16 to 0.98)	1.97/0.03*	0.35 (0.15 to 0.99	1.92 /0.04*	0.4 (0.15 to 1.01	1.92 /0.04*
TC	11(22)	22(44)	21(42)	19(38)	2.5(1.01-6.24)	1.99/0.04*	2.51(1-6.29)	0.47/0.03*	2.75 (0.57 to 4.46)	0.47/0.03*
CC	10(20)	5(10)	7(14)	11(22)	0.63(0.18 to 2.1)	0.75/ 0.45	0.92(0.29 to 2.72	0.89/0.37	1.59(0.3 to 2.82)	0.89/0.37
Allele										
T	69(69)	68	65	59	0.95 (0.52 to 1.73)	0.15/0.87	0.83(0.46 to 1.5)	1.5/0.16	0.64 (0.36 to 1.15)	1.5/0.16
C	31(31)	32	35	41	1.04(0.57	1.47/ 0.89	1.19(0.6	1.47/0.14	1.47/0.14	

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Cytokines (IL27) quantification

Serum levels of IL27 were quantified by enzyme-linked immunosorbent assays (ELISA) using the predesigned kit as per the manufacturer's instructions (R&D Systems, Inc., USA) in all subjects enrolled for the present investigation. We note that there is a clear significance between the measured gene level in the abortion samples with all the totals because the value<0.05 .show in Table 5.

TABLE 5: Comparison serum level of IL-27 among the studied groups (mean±SD)

Biomarkers	Groups	Median (IQR)	Mean±SD	P-value
IL-27	Control First trimester	120.34(103.76-130.65) 199.15 (181.55-215.85)	118.62±16.57 199.34±26.02	0.04*
	Control Second trimester	120.34(103.76-130.65) 181.48 (157.07-196.37)	118.62±16.57 173±32	0.001*
	Control Third trimester	120.34(103.76-130.65) 114.32 (94.98-158.85)	118.62±16.57 119.69±37.3	P<0.001*

Explanations: P-value <0.05 mean significant; NS: non-significant

CONCLUSION

The IL-27 serum concentration elevated with significant differences in recurrent abortion in both groups compared to healthy women, Also, recurrent abortion had significant differences compared to healthy women (P<0.05). Also, in recurrent abortion, the level of IL-27 for, GC, and CC genotypes showed significant differences compared to healthy and pregnant women (P<0.05). Also, in recurrent abortion, the level of IL-27In conclusion, the level of IL-27 in recurrent abortion women with free pathogen was higher than the recurrent abortion women, which may be due to the pro-inflammatory for l27. SNP of IL-27 has been represented as a risk factor in recurrent abortion women.

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