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Original Article

Single Nucleotide Polymorphism of IL-18 (Rs 1946519) in Recurrent Aborted Iraqi Women and Its Association with Toxoplasmosis

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Abstract

Background: We aimed to shed light on the risks of elevated levels of IL-18 in aborted women with toxoplasmosis by evaluating the risk or protective function of alleles or genotypes for single nucleotide polymorphism (SNP) of IL-18 (rs 1946519), which might be related to the susceptibility to toxoplasmosis.

Methods: IL-18 levels in patient and control blood samples were determined using ELISA, and the SNP IL-18 (ra 1946519) was subjected to the high-resolution method.

Results: Compared to healthy pregnant women (HP), the IL-18 serum levels of recurrent abortion with toxoplasmosis (RAWT), recurrent abortion without toxoplasmosis (RAWOT), and healthy non-pregnant (HNP) women decreased with significant differences. Additionally, a strong association between patients and controls was found in the SNP IL-18 data. RAWT and RAWOT with the genotypes AA and AC had significantly lower IL-18 serum levels than women HP, according to the distribution of IL-18 serum levels by SNP.

Conclusion: The serum level of IL-18 varied by genotype in patients with substantial differences compared to controls, while the SNP of IL-18 has been linked as a risk factor in toxoplasmosis-infected recurrent abortion women.

Introduction

Two or more abortions that occur back-to-back before 20 weeks of gestation constitute the multifactorial event known as recurrent pregnancy loss (RPL) (1, 2). 1%-5% of women of reproductive age experience RPL, a significant reproductive issue (3). RPL can result from a variety of factors, including ones that are endocrinological, chromosomal, anatomical, and genetic (4). RPL can occasionally result from immunologic issues (5). A successful pregnancy is correlated with the balance of cytokines produced by Th1 and Th2 cells (6). Additionally, the lowering of cytokines, which are produced by Th1 cells, is linked to the physiological development of the fetus during pregnancy (7). One of the most prevalent illnesses that develop during pregnancy is toxoplasmosis, which is also one of the major causes of morbidity and mortality in fetuses (8).

Healthy pregnancies produce a range of cytokines in the placental and decidual tissue (9). Studies on IFN-ɣ impact on congenital *T. gondii* infection in mice models show that high levels of IFN-ɣ can cause the embryo to resorb without actually infecting the placenta, highlighting the significance of cytokines in the pathogenicity of toxoplasmosis and miscarriage. This shows that *T. gondii* can increase the Th1 or low response of IL-10/TGF β-/IL-27, which might indirectly cause abortion in women.

Several different cells, including macrophages, chondrocytes, and osteoblasts, generate interleukin 18 (IL-, 18) and stimulate cell mediated immunity as a response to bacterial toxins, as well as induced the nuclear factor-*κ*B (NF-*κ*B) and interferon gamma production (IFN-ɣ) (10). The IL-18 gene, also known as IFN-ɣ inducing factor, has six exons situated on chromosome 11q22.2. Exon 2 of the gene contains the translation-starting site (11). The pro-inflammatory cytokine IL-18, which has an 18-kDa molecular weight and is produced by macrophages and monocytes, promotes a variety of inflammatory reactions, including the activation of interferon expression, the maturation of T cells and NK cells, and indirectly through promoting IL-1β expression (12).

Pregnancy problems including preeclampsia have been associated with modifications in IL-18 expression and production (13, 14), premature birth (15), in vitro fertilization embryo transfer failure (16), implantation failure, and recurrent miscarriage (17). Additionally, they have been connected to a higher risk of autoimmune and inflammatory diseases (13). The expression of cytokines may be increased or decreased by functional genetic polymorphisms in the genes of cytokine, which helps to regulate the genetics of inflammatory responses and susceptibility to or resistance to infectious diseases. SNPs help identify variants linked to illness vulnerability.

We aimed to provide helpful information on the risk of elevated levels of IL-18 in aborted women with toxoplasmosis by finding the risk or protective role of alleles or genotypes for SNP of IL-18 (rs 1946519), which may be associated with the vulnerability to toxoplasmosis.

Materials and Methods

The design of the study groups and sample collection

From November 2021 to March 2022, blood samples from the women were taken at the AI-Alawiya Maternity Teaching Hospital and the AL-Yarmouk Teaching Hospital in Baghdad, Iraq. Iraqi women were separated into two primary groups for the study: patients, and controls. These main categories were then divided into four subgroups. The control group consisted of 50 healthy pregnant women (HP) and 50 healthy non-pregnant women (HNP). The two patient groups were 50 recurrent abortion women without toxoplasmosis (RAWOT) and 50 re-

current abortion women with toxoplasmosis (RAWT), both of whom had previously been diagnosed with anti-*Toxoplasma* IgG and IgM. Information about the control and patient groups was gathered using a pre-made questionnaire. Women, who were at least 35 years old, had experienced a certain number of miscarriages and abortions had to meet certain criteria in order to be included.

The study was approved by the Ethics Committees of the Biology Department, College of Science, University of Baghdad in addition to the consent of the participants (Reference No.CSEC/1121/0085)

Measurement of the serum IL-18 level

Following the kits manufacturing instructions (catalog number: EH0011, Wuhan Fine Biotech, China), IL-18 was found in human serum.

Genetic evaluation

Using the *EasyPure*® Blood Genomic DNA Kit, Frozen whole blood was used to extract the DNA, which was then put in an EDTA tube. Single nucleotide polymorphism (SNP) discrimination based on allelic variation. Primers that were created with Primer 3plus, V4 and double-checked by the University Code of Student perform (UCSC) programs and their reference sequences in the National Center for Biotechnology Information (NCBI) database were used to perform research on IL-18 (rs 1946519). Alpha DNA Ltd. (Canada) produced and lyophilized them. Real-time PCR technology (Real-time thermal cycler, QIAGEN Rotor gene Q, Germany) used to estimate SNP.

Statistical analysis

The one-way ANOVA analysis was carried out using Graph Pad Prism version 8.0.1 for Windows (Graph Pad Software, San Diego, California, USA). The results of the serum IL-18 level were presented as the mean's standard deviation (SD), and statistically significant differences were investigated. The odds ratio (OR) was performed using the statistical software epidemiology (WINPEPI) version 11.65, Fischer's exact probability and Chi-square were used to estimate the risk connected to genotypes and alleles. Additionally, *P*-values less than 0.05 are considered statistically significant. By using the HRM technique, the genotype and allele frequencies for SNPs were determined. Using Michael H. Court's online calculator (2005–2008), Each SNP's Hardy-Weinberg equilibrium (HWE) was investigated. The population was consistent if the *P*-value above 0.05.

Results

IL-18's single nucleotide polymorphism (SNP)

Table 1 illustrates the results of the IL-18 genotypic frequencies. Hardy-Weinberg equilibrium-adjusted genotypic distributions of IL-18 (rs1946519 A/C) were seen in the groups. There were significant differences between the genotype CC and mothers age in age <35 yr and overall, with *P*-values of 0.02 and 0.01 respectively. These genotypes did not appear related to the risk of maternal age progression, according to Table 2 distribution of genotypes polymorphism for IL-18 by maternal age. Additionally, the genetic models (codominant, recessive) had significant association between PA and CON groups in the genotypes CC with *P*-value 0.04, 0.004, (OR= 2.69, 3.75 for each respectively).

The genotype AC in the over dominant model had a significant difference between PA and CON groups with *P* value0.0004, OR-0.42 (Table 3). The polymorphic genotypes CC had a significant difference between RAWT and HNP with *P* value 0.02, OR, 6.77. Moreover, in recurrent abortion women without toxoplasmosis, the genotypes AC and CC had a significant difference with *P* value 0.04, 0.02 and OR (0.37 and 6.77 for each respectively) in comparing with HNP women. The result showed the genotype AC has significant differences between RAWOT and HP with *P*-value 0.001, OR, 0.18 (Table 4).

Table 1: The genotype and Hardy-Weinberg Equilibrium (HWE) percentage frequencies of IL-18 (rs 1946519) in the studied groups

Groups			*AA*	*AC*	*CC*	*HWE P>0.05*
Controls (N=100)	Observed	N	36	57	7	0.01
		%	36	57	7	
	Expected	N	41.6	45.8	12.6	
		%	41.6	45.8	12.6	
Healthy women (N=50)	Observed	N	25	23	2	0.13
		%	50	46	4	
	Expected	N	25.2	20.6	4.2	
		%	50.4	41.2	8.4	
Pregnant women (N=50)	Observed	N	13	32	5	0.03
		%	26	64	10	
	Expected	N	16.8	24.4	8.8	
		%	33.6	48.8	17.6	
Patients (N=100)	Observed	N	42	36	22	0.01
		%	42	36	22	
	Expected	N	36	48	16	
		%	36	48	16	
Recurrent abortion (N=50)	Observed	N	27	12	11	0.001
		%	54	24	22	
	Expected	N	21.8	22.4	5.8	
		%	43.6	44.8	11.6	
Recurrent abortion with toxoplasmosis (N=50)	Observed	N	15	24	11	0.8
		%	30	48	22	
	Expected	N	14.6	24.8	10.6	
		%	29.2	49.6	21.2	

Table 2: The genotype distribution of the IL-18 SNP (rs1946519) by maternal age

Age(yr)	*Groups*	*Genotypes*			*Total*
		AA	AC	CC	
Age < 35	Patients	41	33	22	96
	Control	35	46	7	88
	OR	1.07	0.65	2.88	
	CI	0.62 - 1.83	0.38 – 1.12	1.17- 7.07	
	P-value	0.7	0.1	0.02*	
Age ≥35	Patients	1	3	0	4
	Control	1	11	0	12
	OR	3.0	0.81	2.7	
	CI	0.15 – 59.8	0.14 – 4.50	0.04 – 16.9	
	P-value	0.4	0.8	0.6	
Overall	Patients	42	36	22	100
	Control	36	57	7	100
	OR	1.16	0.63	3.14	
	CI	0.69 – 1.97	0.38 – 1.04	1.28 – 7.68	
	P-value	0.5	0.07	0.01**	

Table 3: The co-dominant, dominant, recessive, and over dominant genetic models for IL-18 (rs 1946519) genotypes and allele associations

Genetic model	*Genotype and allele*	*Patients N=100(%)*	*Control N=100(%)*	*OR (CI: 95%)*	*P-value*
Codominant	AA reference	42	36	1.00 (reference)	--
	AC	36	57	0.5 (0.30 - 0.99)	0.06
	CC	22	7	2.69 (1.04 - 6.96)	0.04
Dominant	AA reference	42	36	1.00 (reference)	--
	AC/CC	58	64	0.95 (0.54 - 1.67)	0.8
Recessive	AA/AC reference	78	93	1.00 (reference)	--
	CC	22	7	3.75 (1.53 - 9.20)	0.004
Overdominant	AA/CC reference	64	43	1.00 (reference)	--
	AC	36	57	0.42 (0.24 - 0.75)	0.004
Allele	A reference	120 (60%)	129 (64.5%)	1.00 (reference)	--
	C	80 (40%)	71 (35.5%)	1.2 (0.81 - 1.81)	0.4

PA: Patient; CON: Control; OR: Odds ratio; CI: Confidence interval; *P*-value< 0.05

Table 4: Comparison of genotypes and allele frequency of IL-18 (rs1946519) among the studied groups

Genotype	*HNP*	*HP*	*RAWOT*	*RAWT*	*HNP vs RAWT*		*HNP vs RAWOT*		*HP vs RAWT*		*HP vs RAWOT*	
	N (%)	N (%)	N (%)	N (%)	*P*-value	OR (CI:95%)	*P*-value	OR (CI:95%)	*P*-value	OR (CI:95%)	*P*-value	OR (CI:95%)
AA	23 (46)	13 (26)	27 (54)	15 (30)	0.15	0.5 (0.22-1.13)	0.8	1.2 (0.50-2.77)	0.8	1.2 (0.51-2.90)	0.008	3.34 (1.45-7.69)
AC	25 (50)	32 (64)	12 (24)	24 (48)	1	0.92 (0.42-2.01)	0.04	0.37 (0.16-0.86)	0.2	0.52 (0.24-1.15)	<0.001	0.18 (0.08-0.42)
CC	2 (4)	5 (10)	11 (22)	11 (22)	0.02	6.77 (1.44-31.86)	0.02	6.77 (1.44-31.86	0.2	2.54 (0.82-7.85)	0.2	2.54 (0.82-7.85)
Total	50	50	50	50								
Allele												
A	73 (73)	58 (58)	66 (66)	54 (54)	0.008	O.43 (0.24-0.78)	0.3	0.72 (0.39-1.31)	0.1	0.6 (0.34-1.07)	0.3	1.4 (0.79-2.49)
C	27 (27)	42 (42)	34 (34)	46 (46)	0.008	2.03 (1.28-4.15)	0.3	1.39 (0.76-2.54)	0.1	1.65 (0.94-2.92)	0.3	0.71 (0.67-2.05)
Total	100	100	100	100								

CON: Control; HNP: Healthy non pregnant women; HP: Healthy pregnant women; PA: Patient; RAWOT: Recurrent abortion women without toxoplasmosis; RAWT: Recurrent abortion women with toxoplasmosis; OR: Odds ratio; CI: Confidence interval; *P* value

The relationship between IL-18 serum levels and SNP (rs1946519)

The IL-18 data indicated a statistically significant difference between HP women and the rest groups (*P* <0.05). When compared to HP (116± 41.65 pg/ml), the IL-18 levels of RAWT women, HNP women, and RAWOT decreased to (96.70± 24.04 pg/ml), (82.52± 10.81 pg/ml), and (80.73± 25.90 pg/ml), respectively. Table 5, and Fig. 1 depicts the results of IL-18 serum levels determined by SNP (rs1946519). Compared to the genotypes in the CON groups (110.6 ±36.41, 99.5± 26.37, 88.6 ±94pg/ml, respectively), the serum levels of IL-18 for the AA, AC, and CC genotypes decreased in the PA groups with significant differences (*P*<0.05). There were no variations in genotypes within either group. Moreover, the results showed the serum level of IL-18 in the Table 6 and Fig. 2 for the genotypes AA, AC and CC decreased in HNP , RAWT and RAWOT respectively comparing to the genotypes AA, AC and CC in HP women respectively with significant differences (*P*<0.05). Additionally, there were significant differences between the genotypes AC in RAWOT and the AA, CC in the rest groups.

Table 5: Distributions of serum level for IL-18 pg/ml (Mean± SD) in patients and controls by SNP (rs1946519) genotypes

Groups	*SNPs genotypes*		
	AA	AC	CC
Controls	99.5±26.37A	110.6±36.41B	88.6±18.94A
Patients	88.01±27.60A	89.01±27.92A	89.2±22.46A

Similar letter within each row are non-significant differences (P >0.05).
Different capital letter within each row are significantly different (P<0.05)

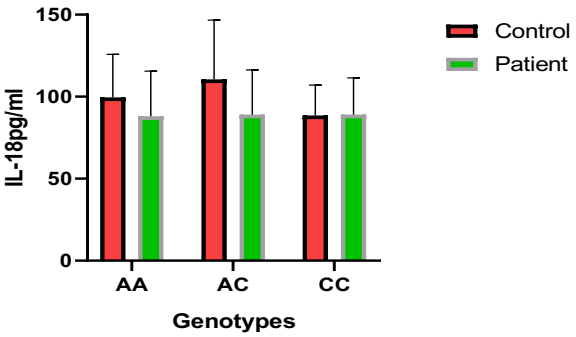

Fig. 1: Distribution of serum IL-18 levels (mean±SD) by SNP genotypes in patients and controls

Table 6: Distributions of serum level for IL-18 pg/ml (Mean±SD) in healthy women, pregnant women, recurrent abortion, and recurrent abortion with toxoplasmosis by SNP (rs1946519) genotypes

Groups	*SNPs genotypes*		
	AA	**AC**	**CC**
Healthy women	82.5 ± 9.76A	86.6 ±11.79A	78.5 ±7.29A
Pregnant women	118.7 ± 36.60A	114. 6 ± 45.04A	116.5 ± 22.91A
Recurrent miscarriage	82.7 ± 25.92A	78.1 ±21.88B	81.5 ± 31.96A
Recurrent miscarriage with toxoplasmosis	97.7 ± 25.49A	98.01 ±27.14A	96.6 ± 15.09A

Similar capital letters within each row are non-significantly different (P>0.05).
Different capital letters within each row are significantly different (P<0.05)

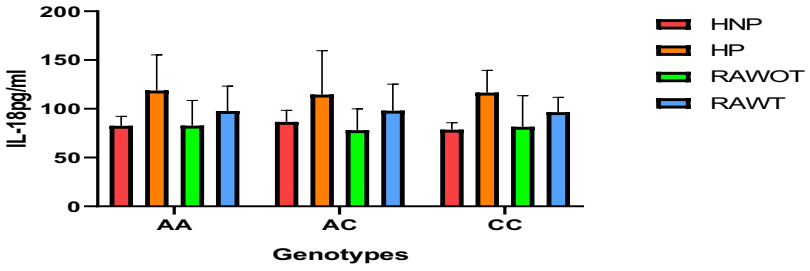

Fig. 2: Distributions of serum IL-18 levels (mean±SD) by SNPs genotypes in HNP, HP, RAWOT, and RAWT

Discussion

The parasite's complexity life and the immune system of the host have been revealed by SNP research, and this knowledge alone would be adequate to explain the interindividual variability in host immunological responses that lead to in different clinical manifestations.

For a more accurate explanation of how genes affect illness susceptibility and the unequivocal identification of variables responsible for causing disease, functional examination of illness-associated polymorphisms is essential. The ability to anticipate the course of a complicated disorder's sickness could be strengthened and more accurately by the discovery of gene-gene interactions (18).

There is a consensus that noncoding polymorphisms influence the expression of nearby genes, which in turn influences the variance and etiology of a characteristic, and that differences in gene expression may be a significant source of phenotypic variability in complex illnesses (19). To study putative regulatory mechanisms mediated by cytokine SNPs, the work has documented allele-specific events by assessing their transcriptional differences in terms of reporter gene activities and unbalanced allelic expression. This result is in line with recent research that discovered a significant connection between IL-18 and RPL (20).

In addition, in the Iranian population, significant differences have been noticed between patients and controls about the frequencies of the CC genotype in the IL-18 gene polymorphism (rs 1946518) (21). Moreover, a case-control study of Bahraini women revealed a correlation between RPL and the (rs 1946518) polymorphism (21). However, no correlation was found between the (rs1946518) polymorphism and RPL in two investigations of Iranian and Solenians women (22). In-depth information on the mechanistic consequences of cis-regulatory variations in managing cytokine gene expression in *Plasmodium falciparum*-mediated malaria was provided by Baso et al (23). That work, however, highlighted the prospect that this complex feature may require much more intricate regulation complexities than initially thought. There are a colossal number of genetic polymorphisms in the human genome. Due to their widespread distribution across a particular genome and comparative affordability, SNPs are the most often employed molecular markers in genetic disease investigations (24). SNPs can change the amino acids that are encoded, remain silent, or appear in non-coding sequences. They can harm proteins, messenger RNA structure (stability), and promoter activity (gene expression). Consequently, locating and examining numerous gene variants may help us better understand how they affect gene function and a person's health (25). In addition, there are several studies were performed recently on pro-inflammatory and anti-inflammatory cytokines role such as IL-3, IL- IL-17A, and IL-27 which are related to pregnancy loss also genetic variants of these cytokines are related to the sensitivity of *Toxoplasma* infection. (26-28) IL-17 is considered a pro inflammatory cytokine, which produced via Th17 cells, NK cells and CD4+ T cells during toxoplasmosis (29). The role of Th-17 in the immune system against toxoplasmosis leads to increase of IL-17 with the increase of T-helper-1(30).Additionally , IL-17 in human toxoplasmosis is expressed via T-helper CD4+ and T-helper CD8+ cells affecting in pregnancy via controlling parasite invasion and replication, which has a role in abortion or fetal malfunction predominantly (31). It was observed that IL-17 is a significant inflammatory biomarker in preeclampsia with useful indication potential to predict the severity of the disease (32). The SNP of IL-17 represented a protective factor associated with genotype AA and allele A in recurrent abortion women with toxoplasmosis and recurrent abortion women, whereas allele G in recurrent abortion women with toxoplasmosis is a risk

factor (26). However, IL-18 concentrations are known to vary through the course of infection and are dependent on the parasite strain and inoculum used. Gorfu et al (33), have suggested that this cytokine plays a pivotal role in mediating acute toxoplasmosis with the cytokine playing an important early role in the control of parasite replication. However, high levels of IL-18 have previously been shown to cause dysregulated induction of prepathological cytokine levels that contribute to lethality in high-dose, virulent infections (34). In this study, the SNP of IL-18 has been linked as a risk factor in toxoplasmosis-infected recurrent abortion

An increased level of some chemokine Ligands is noticed during parasite infection (35). The infection with parasite like cutaneous leishmaniasis is associated with higher levels of IL-8 in patient group in comparison with the control group that the limits cannot afford the study such as the sample must be large, variation in the males and females with different age and different dose (36).

Conclusion

In recurrent abortion with toxoplasmosis and recurrent abortion women, the genotype CC of SNP IL-18 (rs1946519) may be protective, whereas allele A in recurrent abortion women with toxoplasmosis is a risk factor. Additionally, IL-18 levels linked with genotypes AA and AC were found to be rising in cases of recurrent abortion with toxoplasmosis while decreasing in cases of genotype CC, demonstrating the protective nature of this genotype.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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