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Research Article

Distribution of Interleukin-6 Promoter Genetic Variants Among *Toxoplasma gondii*-Infected Pakistani Women

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Abstract

Interleukin-6 (IL-6) is a multifunctional cytokine that plays a key role in infection control and pregnancy maintenance. IL-6 genetic variability affects the pregnancy outcome and susceptibility to *Toxoplasma gondii* (*T. gondii*) infection. This study aims to investigate the prevalence of IL-6 promoter polymorphisms in *T. gondii*-infected women. This study included 163 reproductive-age women, including 83 *T. gondii*-infected women and 80 control women. The blood and placental tissue samples were collected from participants. The DNA was extracted and amplified for *T. gondii* DNA and IL-6 gene promoter polymorphisms. The amplified IL-6 DNA was sequenced through Sanger's sequencing method. The -174 G/C, -373 AnTn Tract, -572 G/C, and -597 G/A polymorphisms were detected in the IL-6 gene promoter. The distribution of the CC genotype (0.49) in antibodies and DNA-positive women, the GG genotype in antibodies-positive and DNA-negative (0.47) and control women (0.56) at -174G/C SNP was significantly high ($p=0.005$). The GG genotype at -572G/C SNP was insignificantly ($P=0.143$) high in antibodies and DNA-positive (0.44), antibody-positive and DNA-negative (0.42), and control women (0.54). The frequency distributions of the AA genotype (0.42) in antibodies and DNA-positive, the GG genotype in antibodies-positive and DNA-negative (0.38), and control women (0.60) at -597G/A SNP were significantly high ($P=0.0012$). Frequencies of the A10T11/A10T11 (0.28) and the A9T11/A9T11 (0.27) genotypes in infected women, and the A8T12/A10T10 and the A10T10/A10T10 genotypes in control women (0.20 each) were significantly high ($P<0.001$). It is concluded that the frequency distributions of IL-6 genotypes at -174G/C, -597G/A, and -373 AnTn SNPs are significantly variable in *T. gondii*-infected women.

Keywords: Interleukin-6, Single Nucleotide Polymorphism, *Toxoplasma gondii,* gene promoter, genetic variation

1. Introduction

Interleukin-6 (IL-6) is a multifunctional acute-phase response mediator cytokine initiating the immune response against pathogens *(Fisch, Clough, and Frickel 2019)* and helps maintain the pregnancy and promote embryo development (Prins, Gomez-Lopez, and Robertson 2012). IL-6 is produced by dendritic cells, myocytes, leucocytes, adipocytes, osteoblasts, chondrocytes, Leydig cells, fibroblasts, keratinocytes, astrocytes, placental trophoblasts, and endothelial cells (Rose-John et al. 2023). It performs many biological actions such as regulation of immune responses, inflammation, hematopoiesis, bone metabolism, embryonic development, and oncogenesis (Hirano 2021). It differentiates and activates macrophages, T-cells, and B-cells, and also regulates CD4+ Treg cells, CD8+ Treg cells, and Th17 cells during an immune response (Cordeiro et al. 2013). *T. gondii* is recognized by toll-like receptors on immune and non-immune cells that activate the release of various cytokines,

including IL-6 (Al-Baldawy, Al-Marsomy, and Kahleel 2022). *T. gondii;* a unicellular parasite of the group protozoa that causes a zoonotic disease known as "toxoplasmosis" in humans. This parasite is cosmopolitan in distribution and medically important because of economic losses. It is estimated that around ~33% of people suffer from this infection globally (Smith et al. 2021). Immune-competent individuals are usually asymptomatic, but symptoms appear in immune-compromised individuals and in the case of congenital toxoplasmosis (Dubey 2021). The severity and risk of congenital toxoplasmosis mostly depend on immunity, genetic background, and the gestational age of the mother at which she gets an infection. The transmission rate of infection increases with the gestation period(Attias et al. 2020).

The IL-6 overproduction has been associated with various disorders (Heinrich et al. 2003), and its changed bioavailability due to genetic variability may contribute to pregnancy complications (Xiuhua Yang et al. 2022). The IL-6 genotypes have been linked with altered IL-6 bioavailability and risk of problems during gestation (Demirturk et al. 2014). The IL-6 gene is on chromosome 7 at position 7p15.3 and has five exons along with four introns (Mostafa, ELshourbagy, and Shahat 2022). Genetic variations may impact a person's vulnerability to various diseases. IL-6 genetic variants may influence gene expression by altering transcription factor attachment and leading to variations in the inflammatory response (Jassem, Jaber, and Shani 2016). Many single-nucleotide polymorphisms in the promoter part of IL-6, such as −597 G/A, −572 G/C, and −174 G/C, have been studied and associated with acute ischemic stroke (Akhter et al. 2019). The IL-6 gene −174 G/C polymorphism was reported to be linked with changed blood IL-6 levels (W Wujcicka et al. 2015). The IL-6 gene AnTn tract variants may change the binding capacity of transcription factors or DNA conformation, which may change blood IL-6 levels (Komatsu et al. 2005). The IL-6 AnTn polymorphism is reported to be associated with rheumatoid arthritis (Ad'hiah et al. 2018), systemic lupus erythematosus (Jeon et al. 2010), chronic periodontitis (Komatsu et al. 2005), and coronary artery bypass graft surgery (Kelberman et al. 2004). There is no published report available on the distribution of IL-6 promoter polymorphisms in women infected with *T. gondii*. Keeping in view the importance of IL-6 in immunity and pregnancy maintenance, and the lack of published data from Pakistan. Therefore, this study aimed to find the distribution of IL-6 promoter genetic variants in Pakistani women infected with *T. gondii*.

2. Materials & Methods

2.1 Study Population

The current study was carried out on a total of 163 reproductive-age women, including 83 women infected with *T. gondii* and 80 healthy women as a control group. The infected women were further divided into two subgroups i) *T. gondii* antibodies and DNA positive women (Ab+ and DNA+, n=43), and ii) *T. gondii* antibodies positive and DNA negative women (Ab+ and DNA-, n=40). The blood and placental tissue samples were collected from women visiting DHQ hospitals in Dir and Peshawar, Khyber Pakhtunkhwa, Pakistan.

2.2 Ethical Approval

The participants were informed about the theme of the study and written consent was obtained from them. The Research Ethics Board, University of Peshawar, Khyber Pakhtunkhwa, Pakistan, formally approved the study.

2.3 Data and Samples Collection

The demographic data were collected through a predesigned questionnaire. The blood samples (about 3mL) were collected from the women who tested positive for *T. gondii* and age-matched healthy women by trained healthcare workers. The samples were centrifuged at a speed of 1500 rpm for a duration of 10 minutes, after which the sera were separated and stored in tubes at -70°C until further use. The placental tissues were also

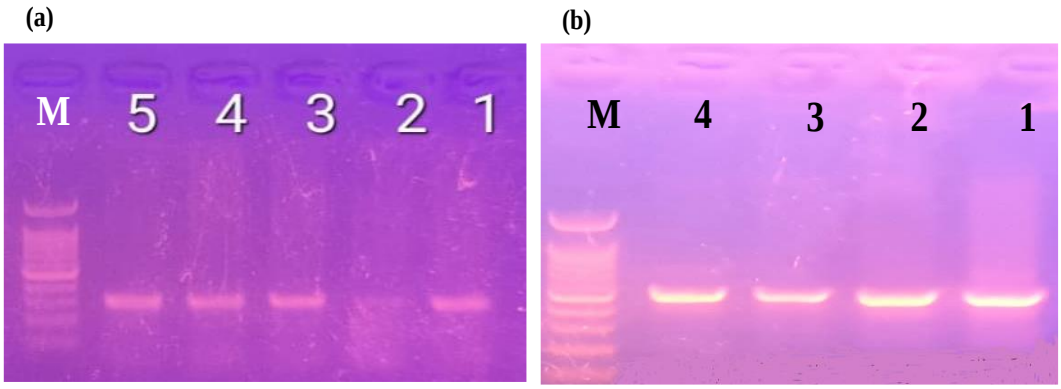

Figure 1: PCR amplification of (a) *T. gondii* B1 gene (287 bp) and (b) IL-6 gene promoter (533 bp). M = 100 bp DNA marker; lanes 1–5 represent DNA samples from study subjects.

collected from women infected with *T. gondii* by the gynecologists for the confirmation of T. gondii DNA.

2.4 DNA Isolation

DNA was isolated from blood and placental tissues through a DNA extraction kit (Gene JET Gel Extraction Kit) according to the manufacturer's guidelines. The extracted DNAs were preserved at −20°C for further processing.

2.5 Amplification of *T. gondii* and IL-6 DNA

DNA isolated from the placental tissues of infected women was amplified for the highly conserved B1 gene of *T. gondii* through conventional PCR, utilizing the methodology specified by (Sardarian et al. 2018). The IL-6 gene's promoter region was amplified using standard PCR techniques as outlined by (Parveen, Shukla, and Agarwal 2013) with appropriate primers and procedures. The *T. gondii* DNA product, measuring 287 base pairs, and the IL-6 gene product of 533 base pairs, were amplified, loaded onto a 2% agarose gel, and visualized using gel documentation (Figure 1).

2.6 DNA Sequencing and Analysis

The amplified products of the IL-6 gene were sequenced using the Sanger sequencing technique. The resulting sequences were verified through the Basic Local Alignment Search Tool. The most recent version of BioEdit software for Windows was employed to examine multiple sequence alignments and identify genetic variations within the sequences.

2.7 Data Analysis

The data were examined utilizing the most recent version of SPSS software, with a P value of less than 0.05 interpreted as statistically significant. The frequencies of genotypes and alleles were evaluated and tested for Hardy-Weinberg equilibrium using the Chi-Square test.

3. Results

3.1 Demographic Data

There was no significant difference (P=0.115) in the mean age (Mean ± SD) of the study participants. The women infected with *T. gondii* had significantly higher contact with cats as compared to the healthy women (p<0.001). The majority of the infected women were illiterate regarding schooling (P=0.001). The infected women positive for *T. gondii* antibodies and DNA had the highest frequency (0.47) of pregnancy loss during the third trimester, while infected women positive for *T. gondii* antibodies and negative for DNA had the highest frequency (0.48) of pregnancy loss during the first trimester (P=0.001, Table 1).

3.2 Detection of *T. gondii* DNA

T. gondii DNA was detected in the placental tissues of 43 (40 IgM+ and 3 IgG+) infected women out of 83 women. No *T. gondii* DNA was detected in the 40 infected women (Only IgG+) by PCR.

Table 1: Demographic Data of the Study Population

Variables		Group A (n=83)		Healthy Control (n=80)	P-Value
		Ab+ and DNA+ (n=43)	Ab+ and DNA- (n=40)		
Age (Year)	Mean ± SD*	32.35 ± 9.65	30.24 ± 7.77	29.28 ± 8.55	0.115
Contact with cats	Yes	29 (0.67)	24 (0.60)	23 (0.29)	< 0.001
	No	14 (0.33)	16 (0.40)	57 (0.71)	
Education	Illiterate	19 (0.44)	13 (0.33)	11 (0.14)	
	Primary	09 (0.21)	07 (0.18)	19 (0.24)	0.001
	Middle	08 (0.19)	09 (0.22)	16 (0.20)	
	Higher	07 (0.16)	11 (0.27)	34 (0.42)	
Pregnancy Loss	1st Trimester	10 (0.23)	19 (0.48)	00 (00)	
	2nd Trimester	13 (0.30)	10 (0.25)	00 (00)	0.001
	3rd Trimester	20 (0.47)	11 (0.27)	00 (00)	

P<0.05 is significant, *S.D = Standard Deviation

3.3 Prevalence of Genotypes and Alleles of IL-6 Gene Promoter Polymorphisms

The polymorphisms detected in the IL-6 gene promoter region were -174 G/C, -373 AnTn Tract, -572 G/C, and -597 G/A. The genotypes and allele frequencies were analyzed for Hardy-Weinberg equilibrium using the Chi-square test.

The genotypes (P=0.005) and alleles (P<0.001) frequencies at -174G/C SNP were significantly variable in all the study groups. The frequency of the -174CC genotype (0.49) was high in the infected women in group Ab+ and DNA+, while the -174GG genotype was more common in the infected women in Ab+ and DNA- group (0.47) and healthy control women (0.56). The rare -174C allele was significantly more common (0.60) in pregnant women in the Ab+ and DNA+ groups. In contrast, the -174G allele was highly prevalent in Ab+ and DNA- (0.62) and healthy control women (0.69). The difference among the genotypes (P=0.143) and alleles (P=0.429) at -572G/C SNP was insignificant in all the study groups. The frequency of -572GG genotype was higher in Ab+ and DNA+ (0.44), Ab+ and DNA- (0.42), and healthy control women (0.54); however, the difference was not statistically significant. The -572G allele was highly prevalent in Ab+ and DNA+ (0.56), Ab+ and DNA- (0.57), and healthy control women (0.59) as compared to the -572C allele (0.44, 0.43, 0.41, respectively).

The genotypes (P=0.0012) and alleles (P<0.001) frequencies at the -597G/A SNP were significantly variable in all the groups. The -597 AA genotype was more common (0.42) in Ab+ and DNA+ women, while the -597 GG genotype was highly prevalent in the Ab+ and DNA- infected women (0.38), and healthy control women (0.60). The frequency (0.59) of the A allele at -597 G/A SNP was significantly high in Ab+ and DNA+ infected women. In contrast, the -597 G allele was more prevalent in the Ab+ and DNA-infected women (0.55), and healthy control women (0.77).

IL-6 gene promoter -373 AnTn tract genotypes (P<0.001) and alleles (P < 0.001) relationship in all the study groups was found to be significantly variable. The frequency (0.28) of the A10T11/A10T11 genotype was high in Ab+ and DNA+ infected women as compared to the frequencies of the same genotype in Ab+ and DNA- infected women (0.22), and healthy control women (0.13). In contrast, the A9T11/A9T11 genotype was highly prevalent in Ab+ and DNA- infected women (0.27), while both the A8T12/A10T10 and A10T10/A10T10 genotypes were more common in the healthy control women (0.20 each). The A8T12/A8T12 genotype was not detected in the infected women but was detected in the healthy control women (0.17). The

Table 2: Prevalence of Genotypes and Alleles of IL-6 Gene Promoter Polymorphisms.

IL-6 Promoter SNPs	Infected Women (n=83)				Healthy Control (n=80)		P Value
	Ab+ and DNA+ (n=43)		Ab+ and DNA- (n=40)				
-174 G/C Genotype	**No.**	**Freq.**	**No.**	**Freq.**	**No.**	**Freq.**	
GG	12	0.28	19	0.47	45	0.56	0.005
GC	10	0.23	12	0.30	21	0.26	
CC	21	0.49	09	0.23	14	0.18	
Alleles							
G	34	0.40	50	0.62	111	0.69	< 0.001
C	52	0.60	30	0.38	49	0.31	
-572 G/C Genotype							
GG	19	0.44	17	0.42	43	0.54	
GC	10	0.23	12	0.30	08	0.10	0.143
CC	14	0.33	11	0.28	29	0.36	
Alleles							
G	48	0.56	46	0.57	94	0.59	0.429
C	38	0.44	34	0.43	66	0.41	
-597 G/A Genotype							
GG	10	0.23	15	0.38	48	0.60	
GA	15	0.35	14	0.35	27	0.34	0.0012
AA	18	0.42	11	0.27	05	0.06	
Alleles							
G	35	0.41	44	0.55	123	0.77	< 0.001
A	51	0.59	36	0.45	37	0.23	
-373 AnTn Genotypes							
A10T11/A10T11	12	0.28	09	0.22	10	0.13	
A9T11/A9T11	06	0.14	11	0.27	14	0.18	
A8T12/A9T11	07	0.16	05	0.13	10	0.12	
A8T12/A10T10	07	0.16	05	0.13	16	0.20	<0.001
A10T10/A10T10	06	0.14	06	0.15	16	0.20	
A10T10/A10T11	05	0.12	04	0.10	00	0.00	
A8T12/A8T12	00	0.00	00	0.00	14	0.17	
Alleles							
A10T11	29	0.34	22	0.27	20	0.12	
A9T11	19	0.22	27	0.34	38	0.24	
A10T10	24	0.28	21	0.26	48	0.30	<0.001
A8T12	14	0.16	10	0.13	54	0.34	

P<0.05 is significant

A10T10/A10T11 genotype was not detected in the healthy control women, while detected in Ab+ and DNA+ (0.12) and Ab+ and DNA- (0.10) infected women. The A10T11 allele was highly prevalent in Ab+ and DNA+ infected women (0.34), the A9T11 allele was common in Ab+ and DNA- infected women, while the A8T12 allele was commonly found in the healthy control women (0.34) (Table 2).

4. Discussion

The current study aimed to investigate the distribution of IL-6 promoter genetic variants in *T. gondii-infected* and healthy control women from Pakistan. IL-6 is a multi-functional cytokine whose

genetic diversity contributes to a change in immune response and pregnancy-related complications (Mousa and Jasim 2021). IL-6 gene promoter −174G/C SNP is investigated in *T. gondii-infected* women from Poland (Wioletta Wujcicka et al. 2018), toxoplasmic retinochoroiditis from Brazil (Cordeiro et al. 2013), spontaneous miscarriage from Germany and Poland (Drozdzik, Szlarb, and Kurzawski 2013), and recurrent miscarriages in women from India (Parveen, Shukla, and Agarwal 2013). IL-6 promoter -174 G/C and -1363 G/T SNPs have been reported in HCV patients from Punjab, Pakistan (Sadiq et al. 2024), but no published data are available to investigate the prevalence of IL-6 highly polymorphic promoter polymorphisms in *T. gondii*-infected women.

It is reported that the prevalence of the IL-6 -174G/C genotypes was significantly variable in toxoplasmic retinochoroiditis patients and controls. The GG genotype at -174G/C SNP was insignificantly prevalent in both the patients and control groups, while the prevalence of the GC genotype was significantly higher in the patients than controls (Cordeiro et al. 2013). (W Wujcicka et al. 2015) and (Mousa and Jasim 2021) also reported high prevalence of the -174 GC genotype in the *T. gondii*-infected cases and the GG genotype in control women. In contrast, (Wioletta Wujcicka et al. 2018), reported a high prevalence of the -174 GG genotype in both the control and healthy individuals. The prevalence of -174 GC and CC genotypes was higher in patients with cervical and ovarian cancer than in healthy controls (Hashemzehi et al. 2021). In the current study, prevalence of the CC genotype was significantly high in T. gondii antibodies and DNA positive women, while the GG genotype was more common in T. gondii antibodies and DNA negative and healthy control women. The frequency of the mutant C allele was significantly high in the *T. gondii* antibodies and DNA-positive women, while the frequency of the wild G allele was notably high in *T. gondii* antibody-positive and DNA-negative women and healthy control women. The research indicates a high prevalence of the C allele at the -174G/C variation in *T. gondii*-infected women, which aligns with previous findings (Mousa and Jasim 2021).

(Akhter et al. 2019) reported a higher frequency rate of the GC genotype at the IL-6 gene -572 G/C SNP in patients with acute ischemic stroke than in the control group from India. Considering the -597 G/A SNP, the frequency distribution of the GG genotype was significantly high, and the frequencies of the GA and AA genotypes were low in both the patients and controls. (Xuan Yang et al. 2014) reported that the CC genotype at IL-6 -572 G/C SNP was highly prevalent in the young ischemic stroke patients and healthy controls. A study conducted in India reported a high prevalence of the C allele at the -572 G/C SNP in deep vein thrombosis patients (Sharma et al. 2018). In contrast to the published reports, in this research, the prevalence of the -572 GG genotype was considerably high in both the infected and healthy women. Considering the -597 G/A SNP in this research work, the AA and GA genotypes were significantly more common in both groups of *T. gondii*-infected women, while the GG genotype was more prevalent in the healthy control women. It has been reported that variations in the IL-6 promoter -373 AnTn tract constructs might change the helical configuration of DNA and influence IL-6 gene expression (Terry, Loukaci, and Green 2000). The A10T11/A10T11 genotype was reported to be common in patients than in normal individuals (Komatsu et al. 2005). In this study, the frequency of the A10T11/A10T11 genotype was also significantly higher in *T. gondii-infected* women than in healthy control women. (Kelberman et al. 2004) reported a significantly high frequency of the A8T12/A9T11 genotype in the patients. In contrast to the finding of (Komatsu et al. 2005), who reported no prevalence of the A9T11/A9T11 genotype in patients in this study, the A9T11/A9T11 genotype was more common in infected women positive for antibodies and negative for DNA. The A8T12/A8T12 genotype

was not detected in the infected women, while detected in the healthy control women.

5. Conclusion

It is concluded that the CC genotype at -174 G/C SNP, AA genotype at -597 G/A SNP, and the A10T11/A10T11 and A9T11/A9T11 genotypes at -373 AnTn tract polymorphism are significantly prevalent in *T. gondii*-infected women. The GG genotypes at -174 G/C, -572 G/C, and -597 G/A SNPs, and the A8T12/A10T10 and A10T10/A10T10 genotypes at -373 AnTn tract polymorphism are significantly more common in the healthy control women.

Data Availability: All data generated or analyzed during this study are included in this article.

Conflict of Interests: The authors declare that they have no known conflict of interest.

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Ethical Approval: The study was carried out in accordance with the Declaration of Helsinki, and formally approved by the Research Ethics Board, University of Peshawar, Khyber Pakhtunkhwa, Pakistan (registry No. REB-04/02-2024).

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