

## Polymorphisms of IL-1B And IL-10 Genes in Abortion Due to Toxoplasmosis

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### ABSTRACT

Ninety whole blood samples were collected from women attended Ibn-abalady Hospital/Baghdad city. 60 samples were taken from aborted women and 30 samples were taken from healthy women as a control group, from period 1<sup>st</sup> October 2022 to 2<sup>nd</sup> may 2023. According the results, the mean age of aborted women who attacked with toxoplasmosis groups were  $31.50 \pm 1.05$  versus  $34.53 \pm 2.09$  of control groups with non-statistical differences ( $P$ -value=0.2). The results also observed the most cases of 3 (30.0%) among aborted women were at the (27-36) years with 27/60 (73.0%) , while the less cases of toxoplasmosis among aborted women were at the age  $> 46$  years with 3/60 (30.0%), These differences statistically were non-significant ( $P$ -value=0.07). The results also observed there were equal cases of toxoplasmosis of women who habited urban and rural regions 30 /60 (66.7%), 30 (66.7%) respectively. the levels of IgM ( $2.01 \pm 0.15$ ,  $0.13 \pm 0.03$ ) and IgG ( $14.24 \pm 0.91$ ,  $0.09 \pm 0.02$ ) were higher in levels among pregnant women than control groups respectively, these differences in the levels was highly significant ( $p \leq 0.001, \leq 0.001$ ) respectively. the levels of IL-1B ( $24.32 \pm 1.04$ ,  $4.64 \pm 0.36$ ) and IL-10 ( $60.67 \pm 3.46$ ,  $5.61 \pm 0.36$ ) were higher in levels among aborted women than control groups respectively, these differences in the levels was highly significant. there were a significant differences in the levels of IgM ( ) among aborted between the duration of pregnancy with  $P$ -value=0.03, Although the the levels of IgG were increased in levl among aborted women during the the first, second and third ( $14.40 \pm 1.41$ ,  $15.16 \pm 1.77$ ,  $12.86 \pm 1.62$ ) above the normal range ( $\geq 1$ ) respectively. there were non significant differences in the levels of both IL-1B and IL-10 among aborted women during the the first, second and third simister although there were increased in the levels of both IL-1B and IL-10.

**Keywords-** Polymorphisms, IL-1B, IL-10 genes, abortion, Toxoplasmosis.

## I. INTRODUCTION

A strong and protective Th1 immune response is elicited by toxoplasmosis, and this response is characterized by the presence of IL-12, IFN- $\gamma$  and IgG2a antibodies. Despite worm burden remains unchanged, co-infected animal shows highly significant helminth fecundities [1]. In the same manner, in the co-infected animals, the response of Th-2 to *H. polygyrus* shows intense decrease in T-cells that express IL-4, IL-5, IL-13 and GATA-3. In addition, the co-infections led to a shortage of eosinophils & low Th2 effector molecule RELM- $\beta$  expression in the intestinal tissues [2]. In a differing manner, the Th1 response to the parasite is not reduced and parasitemia of *T. gondii* is not affected by

concurrent worm infections. It is of importance to say that the specific re-stimulation of *H. polygyrus* to the splenic cells detected the *H. polygyrus*-reactive CD4<sup>+</sup> T cells which produce a huge amounts of IFN- $\gamma$  among the co-infected humans. In AIDS patients, cerebral toxoplasmosis is a significant health complication, usually noticed in the advanced disease stages and may result in mortality and morbidity. Toxoplasmic encephalitis (TE) can appear In 30-40% of HIV-infected people when the prophylaxis strategies are absent [3]. The TE rate can be effectively reduced when appropriate prophylaxis strategies are applied. Thus, an early diagnosis and treatment of *T. gondii* seriously affects the clinical managements of HIV-infected individuals [4]. Moreover, with the entrance of the highly active

antiretroviral therapy (HAART) and its influence on improving the immunological status of AIDS patients lowered the occurrence of TE and TE-associated deaths. Toxoplasmosis may result in more severe progressions when accompanied with some other infections [5]. Patients with AIDS have a very high burden of *T gondii* infections, particularly in sub-Saharan Africa, and stresses the significance of routine surveillance of Toxoplasmosis in all AIDS patients [6]. In HIV-positive persons, serious opportunistic infections are caused by *T. gondii*, which is of a main public health importance since it leads to psychological and physical defects [7]. In pregnant women and AIDS patients, Toxoplasmosis causes severe encephalitis, neurologic disease, and affects the liver, heart, inner ears and eyes (chorioretinitis). It may also cause ocular diseases and cervical lymphadenopathy in-HIV infected immune competent people [8]. Our study aimed to determine the effect of Toxoplasmosis on polymorphisms in gene sequences of IL-1B and IL-10 interleukins.

## II. MATERIAL AND METHODS

Ninety whole blood samples were collected from women attended Ibn-abalady Hospital/Baghdad city. 60 samples were taken from aborted women and 30 samples were taken from healthy women as a control group, from period 1<sup>st</sup> October 2022 to 2<sup>nd</sup> may 2023. The blood samples were divided into two parts, the first part was put in EDTA tubes for hematological investigations to detect the Complete blood count (CBC), and the second part was placed in plane tubes, then centrifuged after clotting at 3000 rpm for 15 minutes to obtain serum, which is stored at -20C until use. Anti-Toxoplasma gondii anti bodies IgM and IgG were Detected by Elisa technique and IL-1B and IL-10 interleukins were determined by Alisa technique. and IL-10 gene was fractionated in 1.5% agarose gel

electrophoresis stained by Ethidium bromide M: 100bp ladder markers. Lanes resembled 961bp PCR product. Gene sequence were detected by sequencer: Primers  
Primer Name Sequence 5`-3` Annealing Temp. (°C)  
Product size (bp) IL1B-F  
TGTAACGACGGCCAGTCCTGGACTCTCATT  
CATTCTAC 60 956 IL1B-R  
CAGGAAACAGCTATGACCTCGAAGAGGTTTG  
GTATCTG IL10-F  
TGTAACGACGGCCAGTAAGTAAGGGACCT  
CCTATCC 961 IL10-R  
CAGGAAACAGCTATGACAGAGCTCCTCCTTCT  
CTAAC.

## III. STATISTICAL ANALYSIS

The SPSS-20 software program (Faculty version) was used for statistical analysis of data, involving the t-test. ( P < 0.05) value was regarded significant.

## IV. RESULTS

Results documented that the mean age of aborted women who attacked with toxoplasmois groups were 31.50 ±1.05 versus 34.53±2.09 of control groups with non-statistical differences (P-value=0.2). The results also observed the most cases of 3 (30.0%) among aborted women were at the (27-36) years with 27/60 (73.0%) , while the less cases of toxoplasmosis among aborted women were at the age > 46 years with 3/60 (30.0%),These differences statistically were non-significant (P-value=0.07). The results also observed there were equal cases of toxoplasmosis of women who habited urban and rural regions 30 /60 (66.7%),30 (66.7%) respectively, The (P-value=1.0) as arranged in table 1.

Table 1: Distribution the Cases (n=60) and controls (n=30) according to age range (Years)

Age	Case (n=60)	Control (n=30)	Total	P-value
Age (M±SE)	31.50 ±1.05	34.53±2.09	-	t-test=1.29,P-value=0.2 (N.S)
(17-26)	18(69.2%)	8(30.8%)	26(100.0%)	Chi-sequare=6.97 P-value=0.07 (N.S)
(27-36)	27(73.0%)	10(27.8%)	37(100.0%)	
(37-46)	12(70.6%)	5(29.4%)	17(100.0%)	
>46	3(30.0%)	7(70.0%)	10(100.0%)	
Residency	Urban	30(66.7%)	15(33.3%)	Chi-sequare=0.0 P-value=1.0 (N.S)
	Rural	30(66.7%)	15(33.3%)	

The present findings showed that the levels of IgM (2.01±0.15, 0.13±0.03) and IgG (14.24±0.91, 0.09±0.02) were higher in levels among pregnant

women than control groups respectively, these differences in the levels was highly significant (p ≤0.001,≤ 0.001) respectively as arranged in Table- (2).

Table 2: Levels of positive Toxoplama IgM and pHepatitis C virus IgM among aborted women and control groups.

Test	Groups	Mean	SE	T-test	P-value
Toxoplama	Cases	2.01	0.15	12.07	≤0.001

IgM	Controls	0.13	0.03		(H.S)
Toxoplama	Cases	14.24	0.91	15.41	≤0.001
IgG	Controls	0.09	0.02		(H.S)

The results showed that the levels of IL-1B (24.32±1.04, 4.64±0.36) and IL-10 (60.67±3.46, 5.61±0.36) were higher in levels among aborted women

than control groups respectively, these differences in the levels was highly significant ( $p \leq 0.001, \leq 0.001$ ) respectively as showed in table (3).

**Table 3: Levels IL-1B and IL-10 among aborted women and control groups**

Test	Groups	Mean	SE	T-test	P-value
IL-1B	Cases	24.32	1.04	17.87	≤0.001 (H.S)
	Controls	4.64	0.36		
IL-10	Cases	60.67	3.46	15.81	≤0.001 (H.S)
	Controls	5.61	0.36		

The present study showed that there were a significant differences in the levels of IgM among aborted between the duration of pregnancy with P-value=0.03, Although the the levels of IgG were increased in levls among abrted women during the the

first, second and third (14.40±1.41, 15.16±1.77, 12.86±1.62) above the normal range ( $\geq 1$ ) respectively these differences statistically were non significant (P-value=0.6) as arranged in table (4).

**Table 4: Levels of IgM and IgG among abrted women (n=60) during simsiters of pregnancy**

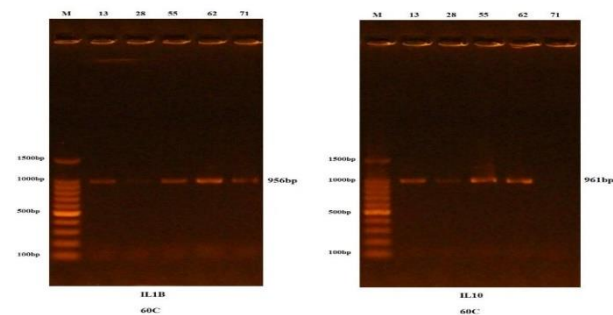
Duration	N	Toxoplasma IgM	P-value	HCV IgM	P-value
		Mean± SE		Mean± SE	
1st	24	1.56±0.10	0.03 (S)	14.40±1.41	0.6 (N.S)
2st	20	2.45±0.37		15.16±1.77	
3st	16	2.13±0.22		12.86±1.62	

The study showed that there were non significant differences in the levels of both IL-1B and IL-10 among aborted women during the the first,

second and third simister although there were increased in the levels of both IL-1B and IL-10 ( $\geq 10$ ), The (P-value=0.73,0.45) respectively as arranged in table (5).

**Table 5: Levels of IL-1B and IL-10 among abrted women (n=60) during simsiter of pregnancy**

Duration	N	IL-1B	P-value	IL-10	P-value
		Mean± SE		Mean± SE	
1st	24	23.47±1.77	0.73 (N.S)	55.83±5.14	0.45 (N.S)
2st	20	24.36±1.72		61.75±6.06	
3st	16	25.55±1.96		66.58±7.27	



**Amplification of IL1B specific regions of the human species have been fractionated onto 1.5% agarose gel electrophoresis stained by Ethidium bromide. M: 100bp ladder markers. Lanes resembling 956bp product & IL-10 specific regions of human sample**

species have been fractionated onto 1.5% agarose gel electrophoresis stained by Ethidium bromider. M: 100bp ladder markers. Lanes resembling 961bp PCR product.

The mutation occurrence for analysis of IL -1B GENE ID 35553 rs20417 NSP rs 16944 gene with wild TT CC and the variation T>C, C>T. Genetic variation of SNPs in the *IL-1B* gene is reported to be capable of creating Toxoplasma infection favoring SNP rs20417 has association with Toxoplasmosis. The changes occurred in nucleotides TT to CC, CC to TT, TC to CT, TC to CC, CC to TT, CC to TT, TC to TC, CC to TT and TT to CC of IL-1B gene sequence.

Table (6) showed the polymorphism occurrence in IL -1B GENE ID 35553, Wild TT CC and the Variation T>C, C>T compared to healthy control.

IL -1B GENE ID 35553		
SNPs	rs 16944	rs 1143627
Wild	TT	CC
Change	T>C	C>T
1	TT	CC
2	CC	TT
3	CC	TT
4	TC	CT
5	TC	CC
6	CC	TT
7	CC	TT
8	TC	CT
9	TT	CC
10	TT	CC
C1	TC	CC
C2	CC	TT
C3	CC	TT
C4	TC	CT
C5	CC	TT

The mutation occurrence for analysis of IL-10 GENE ID 3586 rs20417 NSP rs 16944 gene with wild TT AA and the variation T>C, A>C. Genetic variation of SNPs in the *IL-10* gene is reported to be capable of

creating Toxoplasma infection favoring SNP rs20417 has association with Toxoplasmosis, the change occurred only in the nucleotide TC to AC, of IL-10 gene sequence.

Table (7) showed the polymorphism occurrence in IL-10 GENE ID 3586, Wild CC GG and the Variation T>C A>C compared to healthy control.

IL-10 GENE ID 3586		
SNPs	rs 16944	rs 1143627
Wild	TT	AA
Change	T>C	A>C
1	CC	CC
2	CC	CC
3	TC	AC
4	CC	CC
5	CC	CC
6	CC	CC
7	CC	CC
8	CC	CC
9	CC	CC
10	CC	CC
C1	CC	CC
C2	TC	AC
C3	CC	CC
C4	CC	CC
C5	CC	CC

## V. DISSUSION

The results documented that the mean age of aborted women who attacked with toxoplasmosis groups were 31.50 ±1.05 versus 34.53±2.09 of control groups with non-statistical differences (P-value=0.2). The results also observed the most cases of 3 (30.0%) among

aborted women were at the (27-36) years with 27/60 (73.0%). These findings agreed with ( Najeeb, et al., 2022) who reported that the seroprevalences of anti *T.gondii* antibody IgG or IgM were 62.07% (54/87) & 14.94% (13/87), consecutively. Serum laminin demonstrated significant decreased levels in 1-5 month aborted-women having chronic & acute Toxoplasmosis compared to the controls. It can be concluded that the

laminin levels in females infected with Toxoplasmosis can have an essential roles in early toxoplasmosis diagnoses particularly at the 1<sup>st</sup> five months of pregnancy or abortion since laminin is essential glycoprotein within extracellular matrix components, engaged in embryogenesis, implantations as well as placentations [9]. The levels of IL-1B (24.32±1.04, 4.64±0.36) and IL-10 (60.67±3.46, 5.61±0.36) were higher in levels among aborted women with Toxoplasmosis than control groups respectively, these differences in the levels was highly significant ( $p \leq 0.001, \leq 0.001$ ) respectively. (PASVENSKAITE1, et al., 2021) Significant correlations were determined between IL-10 rs1800871 variant and advanced stages of LSCC patient's group among codominant, recessive & additive models (OR=0.473,  $p=0.027$ , OR=0.510,  $p=0.040$  = and OR=0.733,  $p=0.037$ ). Significant IL-10 rs1800872 variants have been identified in codominant, recessive & additive models (OR=0.473,  $p=0.027$ , OR=0.510,  $p=0.040$  and OR=0.733,  $p=0.037$ ). The SNPs of IL-10 genotype distributions had no effect on the survival rates of LSCC patients (consecutively  $p=0.952$ ,  $p=0.952$ , and  $p=0.991$ ). It can be concluded that there is an association between IL-10:rs1800871 & rs1800872 SNPs and the advanced LSCC stages. The IL-10 SNPs genotypic distribution has no effect on the LSCC patients' survival rate [10]. These cytokines are secreted by the activated macrophage as proproteins, which are proteolytically processed to their active forms by caspase-1 (CASP1/ICE). The mutation occurrence for analysis of IL-1B GENE ID 35553 rs20417 NSP rs 16944 gene with wild TT CC and the variation T>C, C>T. Genetic variation of SNPs in the *IL-1B* gene. These results were matched with ( ) who explained that cytokines are important mediators for inflammatory responses, and are included in various cellular activities, such as cellular differentiations, proliferations as well as apoptosis. The cyclooxygenase-2 (PTGS2/COX2) induction via such cytokine in the CNS is observed to participate in inflammatory pains hypersensitivity. In the same manner, IL-1B was involved in the pathogenesis of human osteoarthritis. Patients severely infected with (COVID-19) show high pro-inflammatory cytokine levels e.g. IL-1B in specimens of bronchio-alveolar lavages fluids. The damage of lungs by (SARS-CoV-2) virus to a large extent, results from inflammatory responses promoted by cytokines like IL-1B. The cytokine genes cluster on chromosome-2 is formed by this gene & 8 other interleukin-1 family gene [11]. The mutation occurrence for analysis of IL-10 GENE ID 3586 rs20417 NSP rs 16944 gene with wild TT AA and the variation T>C, A>C. Genetic variation of SNPs in the *IL-10* gene is reported to be capable of creating Toxoplasma infection favoring SNP rs20417 has association with Toxoplasmosis, the change occurred only in the nucleotide TC to AC, of IL-10 gene sequence. This report agreed (National center 2023) who reported that the gene encodes for an anti inflammatory

cytokine which belongs to class-2 cytokine family. The cells of both adaptive and innate immune system secrete the encoded protein, and is important to limit immune responses to a wide range of pathogenic agents. It was also observed to suppress autoimmune response [12]. The immunosuppressive signal of this protein is mediated by a specific interleukin-10 receptor complex. This gene's aberrant functioning is related to many immune diseases such as graft-versus-host disease, increased susceptibility to HIV-1 infection as well as rheumatoid arthritis. The changes occurred in nucleotides TT to CC, CC to TT, TC to CT, TC to CC, CC to TT, CC to TT, TC to TC, CC to TT and TT to CC of IL-1B gene sequence. These results were in a harmony with (Albosale and Mashkina, 2019) who illustrated that the haplotype -511C/-31T for IL-1 $\beta$  gene is associated with a protective effect against increasing HPV viral load. The frequencies of -511T allele and -511TT genotype of the IL-1 $\beta$  -511C>T were significantly higher among women with HPV in comparison to control group [13]. According to the results; it was found that there is a significant change in the genetic sequence of the interleukin-beta gene in several locations on the spike, and this indicates the effect of Toxoplasma on the sequences of this gene due to the invasion of this parasite and the occurrence of genetic mutations (Salman,2022) [14].

## VI. CONCLUSIONS

We concluded that a very effective impact on the levels of interleukins IL-1B and IL-10 concentrations in the sera of patients, as well as the presence of noticeable mutations in the genetic sequence of IL-1B, and a partial change in the sequences of IL-10.

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