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Original article

Molecular study of TNF-α, IL-1B and IL-10 genes in Eris Covid-19 patients

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ABSTRACT

Background: The combination between exposure to *T. gondii* parasite and COVID-19 infection developed as an important focusing of the present study. **Aim:** This study aims to investigate the polymorphisms of TNF-α, IL-1B and IL-10 genes in Eris Covid-19 patients associated with Toxoplasmosis. **Methods:** This study included (100) patients (52 males and 48 females) infected with Eris (EG.5) Virus, whose ages ranged from (11 to >55) years. The study samples were collected from Al-Numan Hospital in Baghdad City during the period from July 2024 to August 2025. The study also included (50) apparently healthy individuals (25 males and 25 females) whose ages ranged from (11 and >50) as a control group. **Results:** The levels of IL-10 (pg/ml) was higher among patient with *T. gondii* IgM levels >1 (IU/ml) than *T. gondii* IgM levels <1 (IU/ml) with (10.59±2.18, 10.40±2.47), with a non-significant difference (P=0.77), while the levels of TNF-α (pg/ml) was higher among *T. gondii* IgM levels <1 (IU/ml) than *T. gondii* IgM levels >1 (IU/ml) with (19.37±8.41, 13.79±4.96) respectively, with a significant difference (P= 0.002). **Conclusion:** The results revealed there were a highly levels of TNF, IL-1ß and IL-10 concentrations with Eris Coved 19 co-infection with Toxoplasmosis. Also, the results found a variation occurrence in IL-1β, IL-10 and TNFα genes.

Introduction

COVID-19 is gaining momentum again in the United States, with the latest subvariant, EG.5 (Eris), driving a surge in cases and hospitalizations across the country [1]. According to the latest variant estimates from the Centers for Disease Control and Prevention (CDC), EG.5 accounts for the majority of COVID-19 cases in the United States, representing just over 20% of all cases. The world's public health crisis was triggered by COVID-19 pandemic, emphasizing the necessity of understanding the interaction complexities between the immune system and different pathogenic agents [1]. Therefore, the existence of a co-infection which can affect the immune responses during SARS-CoV-2 infections like malaria, dengue and toxoplasmosis, has become obvious [2, 3].

The intracellular parasite *Toxoplasma gondii (T. gondii)* causes toxoplasmosis which is transmitted via oocysts. The most common means of transmission is zoonotic, then comes the congenital transmissions [4]. The infection prevalence differes by regions, and the highest rate was recorded in Africa (61.4%), Oceania (38.5%) then South America (31.2%) [5]. It was estimated that the prevalence rate in pregnant women was (33.8%), with more common in South America (56%) and in

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lower economic regions and less human development index [6]. These factors made *T. gondii* a parasite with global distribution, and despite the rareness of its clinical features, patients may present with fatigue, fever, myalgia, cervical lymphadenopathies as well as ocular involvements in congenital forms [7].

It is important to mention that *T. gondii* and SARS-CoV-2 have the ability for innate immunity stimulation by the use of similar pathways. Infact, both *T. gondii* and SARS-CoV-2 are able activate the toll-like receptors (TLR-s), like TLR-2, TLR-4 as well as and TLR-7, via canonical pathways. Moreover, patients with toxoplasmosis who produce some cytokines can aggravate COVID-19 severity [8].

The combination between exposure to *T. gondii* parasite and COVID-19 infection developed as an important focusing of the present study. The collected relevant data were analyzed via a meta-analysis and a comprehensive systematic review. The present process highlighted the exposure frequency, thereby, it provided a perfect picture of immune responses in patients with both infections [9].

This work will lay a groundwork for forthcoming studies, that enriches scientific understandings of clinical care for patients with COVID-19. Examination of the possible co-existence of other infections, like toxoplasmosis, demonstrates an important implication that can affect such patient's diagnosis and treatment [10]. Our study to determination of polymorphisms of TNF-α, IL-1B and IL-10 genes in Eris Covid-19 patients assotiated with Toxoplasmosis. This study aims to investigate the polymorphisms of TNF-α, IL-1B and IL-10 genes in Eris Covid-19 patients associated with Toxoplasmosis.

Materials and methods

This study included (100) cases (52 males and 48 females) infected with Eris (EG.5) Virus, whose ages ranged from (11 to >55) years. The study samples were collected from Al-Numan Hospital in Baghdad City during the period from July 2024 to August 2025. The study also included (50) apparently healthy individuals (25 males and 25 females) whose ages ranged from (11 and >50) as a control group. This focus was on people who had symptoms of coronavirus and were then confirmed to have the Eris (EG.5) variant. From all participants, (6) ml of blood were collected and put in EDTA and disposable tubes. The disposable tubes were left at room temperature for about 15– 30 minutes, then centrifuged at 3000 rpm for 5 minutes to get serum. Determination of anti-IgM and anti-IgG antibodies of Eris (EG.5) was done by automated fluorescent immunoassay system (AFIAS) Technique. The presence of toxoplasmosis co-infection is determined through serological (ELISA) testing. Then measurement and analysis of gene expression levels of miRNA, and TNF-α was carried out using RT-qPCR techniques. The correlation between these molecular markers and the clinical status of Eris (EG.5) variant, along with the presence or absence of toxoplasmosis co-infection forms were the core analysis of the study. Detectin of TNF, IL-10 and IL-1ß genes was done by conventional PCR technique and sequenced by sanger sequener the primer used:

Ethical approval:

Before beginning this study, all participants provided written consent. Medical ethics approval certification, ethics committee approved the study on number 68/ 318 on Jenuary 11, 2025.

Statistical Analysis

Data analysis was performed using the statistical package of IBM SPSS-22, Chicago, IL, USA). Data of current study were analyzed by Chi-sequare test or using fisher exact probability (F.E.P) test to compare between percentages (qualitative data). T-test was used to compare between two numeric variables. P-Value of ($P<0.05$) was considered as statistically significant (S).

Results

The results showed that the mean age of cases was (37.49±16.19) years versus (41.10±15.94) of the control group, with a non-significant difference (P= 0.19). The results also showed that the age group in the second to fourth decade (26-40) years included 36 (24.0%) cases of Eris (EG.5) out of 100, which was higher than other age groups, followed by the age group (11-25) years and (41-55) years 26 (17.3%) and 22 (14.7%) cases of Eris (EG.5) out of 100 cases respectively. While fewer cases of Eris (EG.5) were 16 (10.7%) out of 100 cases observed in the age group >55 years, compared to the control group. These differences were statistically non-significant (P=0.58). The males infected with Eris (EG.5) were shown to be higher than females with 52 (34.7%) and 48 (32.0%) respectively, with a nosignificant difference

(P=0.81). The results of residency showed equal cases of Eris (EG.5) infection 50 (33.3%) recorded in both urban and rural areas with non-significant difference (P= 0.87) as shown in table (1).

The results demonstrated that the levels of Eris-IgM (IU/ml) was higher in patient group with *T.gondii* IgM levels >1 (IU/ml) than the patients group with *T.gondii* IgM levels <1 (IU/ml) with (2.35±1.12, 1.85±1.01) respectivelly, with a non-significant difference (P=0.14). The level of Eris-IgG (IU/ml) was higher in patients with *T.gondii* IgM levels >1 (IU/ml) than patients with *T.gondii* IgM levels <1 (IU/ml) with (15.08±8.23, 14.20±6.54) respectivelly, with a non-significant difference (P=0.71). Also, the level of IL1-β (pg/ml) was higher in patients with *T.gondii* IgM levels <1 (pg/ml) than patients with *T.gondii* IgM levels >1 (IU/ml) with (24.27±8.45, 23.85±8.27) respectivelly, with a non-significant difference (p=0.86), while the level of IL-10 (pg/ml) was higher in patients with *T.gondii* IgM levels >1 (IU/ml) than patients with *T.gondii* IgM levels <1 (IU/ml) with (10.59±2.18, 10.40±2.47) respectivelly, with non-significant difference (P=0.77), while the level of TNF-α (pg/ml) was higher in patients with *T.gondii* with IgM levels <1 (IU/ml) than patients with *T.gondii* IgM levels >1 (IU/ml) with (19.37±8.41, 13.79±4.96) respectivelly, with a significant difference (P=0.002) as shown in table.(2).

The levels of Eris-IgM (IU/ml) was higher in patients with *T.gondii* IgG levels >1 (IU/ml) than patients with *T.gondii* had IgG levels <1 (IU/ml) with (2.10±1.09, 1.79±0.98) respectivelly, with a non-significant difference (P=0.14) and the levels of Eris-IgG (IU/ml) was higher in patients with *T.gondii* IgG levels <1 (IU/ml) than patients who had *T.gondii* IgG levels >1 (IU/ml) with (14.88±6.62, 13.61±6.96) respectivelly, with no significant difference (P= 0.45), while the levels of IL1-β (pg/ml) was inceased in patients with *T.gondii* IgG levels >1 (IU/ml) than patients with *T.gondii* IgG levels <1 (IU/ml) with (26.41±9.32, 22.48±7.19) respectivelly, with a significant difference (P=0.02). Also, the levels of IL10 (pg/ml) was inceased in patients who had *T.gondii* IgG levels >1 (IU/ml) than patients with *T.gondii* who had IgG levels <1 (IU/ml) with (10.86±2.13, 10.08±2.59) respectivelly, with a significant difference (P=0.10), whereas the levels of TNF-α (pg/ml) was inceased in patients with *T.gondii* IgG levels >1 (IU/ml) than patients with *T.gondii* who had IgG levels <1 (IU/ml) with (19.23±9.25, 18.07±7.38) respectivelly, with a significant difference (P=0.49) as demonstrated in table (3).

Table (4) and figure (4) revealed that a variation occured in rs767455 SNP to TNF GENE ID 7132, theat AA was changed to AA to GG in samples number 9,11 and 15 with Eris cocid 19 co-infection with Toxoplasmosis.

Table (5) and figure (5) showed that a variation occured in rs16944 SNP to IL1B GENE ID 3553 that TT was changed to CC in samples number 1,9 and 10. While there was a varation occured in rs1143627 SNP of IL1B GENE ID 3553 changed from CC to TT in samples number 1,9,10 and 13 in Eris Covid 19 co-infection with Toxoplasmosis.

Table 6 and figure 6 showed that a variation occured in rs1800871 SNP to IL10 GENE ID 3586 that TT was changed to CC in samples number 1,4,,7, 9,10,11,14 and 15. Also there was a varation occutred in rs1800872 SNP of IL1B GENE ID 3553 changed from CC to TT in samples number 1,4,,7, 9,10,11,14 and 15.

Table 1. Demographical picture of the study groups.

Variable		Groups		Total	P-value
		Case (n=100)	Control (n=50)		
Age (M±SD)		37.49±16.19	41.10±15.94		0.19
Age range (Years)	(11-25)	26 (17.3%)	8 (5.3%)	34 (22.7%)	0.58
	(26-40)	36 (24.0%)	20 (13.3%)	56 (37.3%)	
	(41-55)	22 (14.7%)	13 (8.7%)	35 (23.3%)	
	>55	16 (10.7%)	9 (6.0%)	25 (16.7%)	
Total		100 (66.7%)	150 (100.0%)	150 (100.0%)	0.81
Gender	Male	52 (34.7%)	77 (51.3%)	77 (51.3%)	
	Female	48 (32.0%)	25 (16.7%)	73 (48.7%)	
Total		100 (66.7%)	50 (33.3%)	150 (100.0%)	
Residency	Urban	50 (33.3%)	26 (17.3%)	76 (50.7%)	0.87
	Rural	50 (33.3%)	24 (16.0%)	74 (49.3%)	
		100 (66.7%)	50 (33.3%)	150 (100.0%)	

Table 2. The mean levels of Eris antibodies, IL1-β, IL-10 and TNF-α co-infection with Toxopalsmosis.

Parameteres	IgM Categories	N	M±SD	P-value
Eris-IgM	<1	86	1.85±1.01	0.14
	>1	14	2.35±1.12	
ErisIgG	<1	86	14.20±6.54	0.71
	>1	14	15.08±8.23	
IL1-β	<1	86	24.27±8.45	0.86
	>1	14	23.85±8.27	
IL-10	<1	86	10.40±2.47	0.77
	>1	14	10.59±2.18	
TNF-α	<1	86	19.37±8.41	0.002
	>1	14	13.79±4.96	

Table 3. The mean levels of Eris antibodies, IL1-β, IL-10 and TNF-α co-infection with oxopalsmosis.

Parameteres	IgG Categories	N	M±SD	P-value
ErisIgM	<1	56	1.79±0.98	0.14
	>1	44	2.10±1.09	
ErisIgG	<1	56	14.88±6.62	0.35
	>1	44	13.61±6.96	
IL1-β	<1	56	22.48±7.19	0.02
	>1	44	26.41±9.32	
IL-10	<1	56	10.08±2.59	0.10
	>1	44	10.86±2.13	
TNF-α	<1	56	18.07±7.38	0.49
	>1	44	19.23±9.25	

Table 4. The sequence of rs767455 SNP to TNF GENE ID 7132.

TNFRSF1A GENE ID 7132	
SNPs	**rs767455**
Wild	**AA**
Variation	**A>G**
Samples	
1	AA
2	AA
3	AA
6	AG
7	AG
8	AA
9	GG
10	AA
11	GG
12	AA
13	AG
14	AA
15	GG
C1	AG
C2	AG
C3	AG
C4	AG
C5	AA

Table 5. The sequence of rs16944 and rs1143627SNPs IL1B GENE ID 3553.

IL1B GENE ID 3553		
SNPs	**rs16944**	**rs1143627**
Wild	**TT**	**CC**
Variation	**T>C**	**C>T**
Samples		
1	**CC**	**TT**
2	**TC**	**CT**
3	**TC**	**CT**
4	**CC**	**CC**
7	**TC**	**CT**
8		
9	**CC**	**TT**
10	**CC**	**TT**
11	**TC**	**CT**
12	**TC**	**CT**
13	**TC**	**TT**
14	**TC**	**CT**
15	**TT**	**CC**
C1	**TT**	**CC**
C2	**TC**	**CT**
C3	**TC**	**TT**
C4	**TC**	**TT**
C5	**TC**	**CT**

Table 6. The sequence of rs1800871 and rs1800872 SNPs of IL10 GENE ID 3586.

IL10 GENE ID 3586		
SNPs	**rs1800871**	**rs1800872**
Wild	**TT**	**AA**
Variation	**T>C**	**A>C**
Samples		
1	**CC**	**CC**
2	**TC**	**CA**
3	**TC**	**CA**
4	**CC**	**CC**
5	**TC**	**AA**
7	**CC**	**CC**
8	**TC**	**CA**
9	**CC**	**CC**
10	**CC**	**CC**
11	**CC**	**CC**
13	**TC**	**CA**
14	**CC**	**CC**
15	**CC**	**CC**
C1	**CC**	**AC**
C2	**CC**	**AA**
C3	**CC**	**AC**
C4	**CC**	**AA**
C5	**CC**	**aC**

Figure 1. The amplification of rs7674 to TNF gene sepecific region of human blood samples species were fractioned on 2% agarose gel electrophoresis staind with Eth.Br. M: 100bp llader marker. Lanes 1-C4 resemble 664bp PCR products.

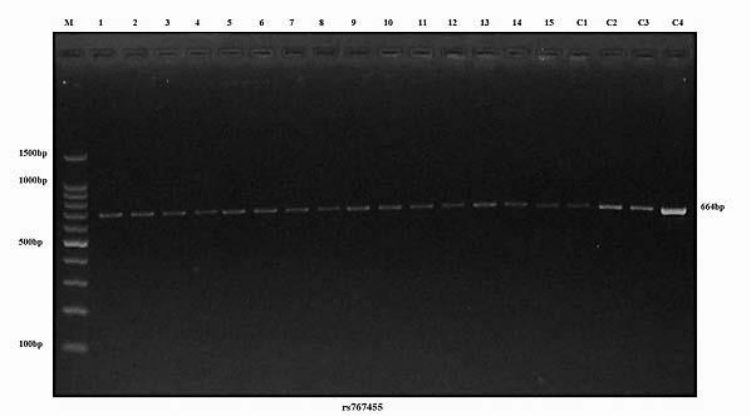


Figure 2. The amplification of rs7674 to IL-10 gene sepecific region of human blood samples species were fractioned on 2% agarose gel electrophoresis staind with Eth.Br. M: 100bp llader marker. Lanes 1-C4 resemble 961bp PCR products.

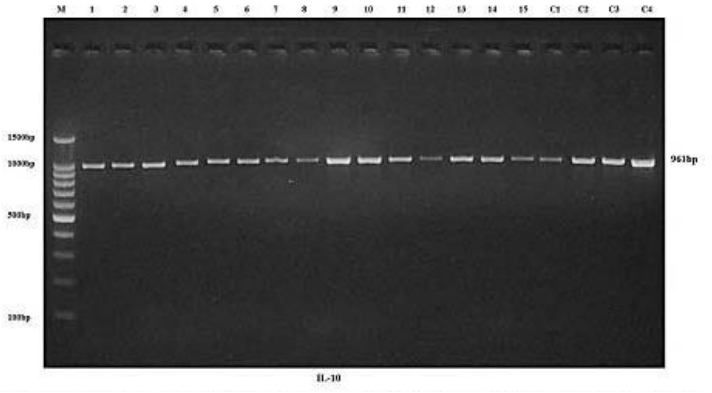


Figure 3. The amplification of rs7674 to IL-1β gene sepecific region of human blood samples species were fractioned on 2% agarose gel electrophoresis staind with Eth.Br. M: 100bp llader marker. Lanes 1-C4 resemble 956bp PCR products

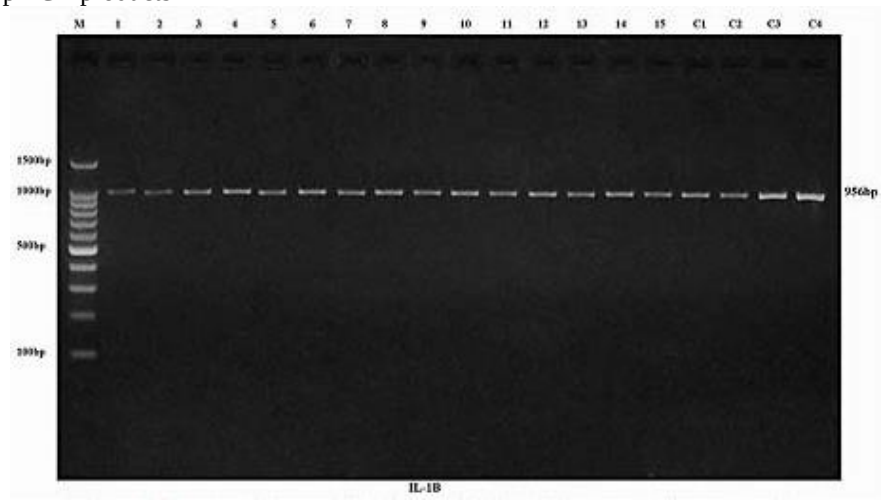


Figure 4. Analysis of SNP rs767455 of TNFRSf1A gene using sagner sequencing. Single "A" Peak indicative of an A homozygous allele. Sinlge "G" peak indicative of G homozygous allele. Presence of the "A" and "G" peak indicative A/G hetrozygous allele.

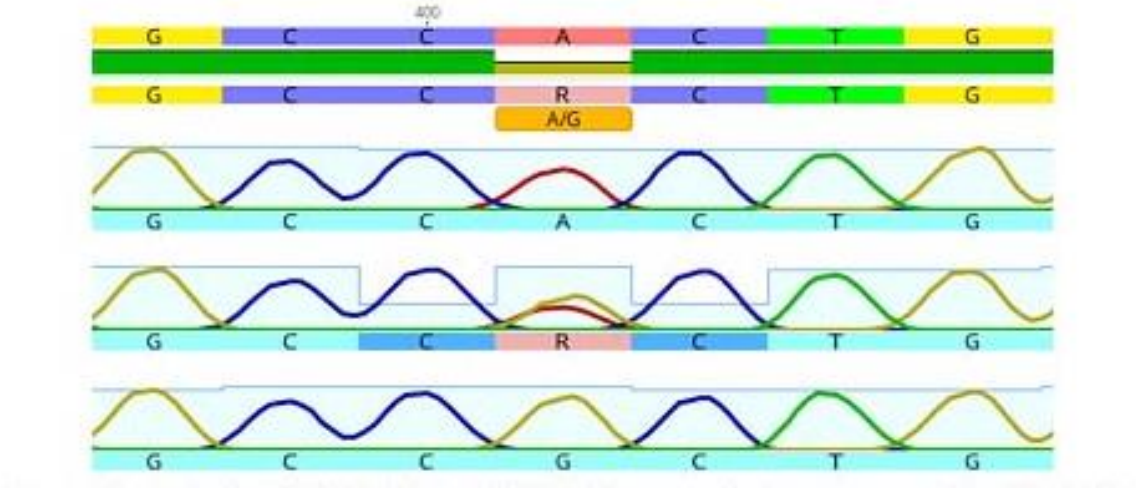


Figure 5. Analysis of SNP rs16944 of IL-1β gene using sagner sequencing. Single "T" Peak indicative of a T homozygous allele. Sinlge "C" peak indicative of C homozygous allele. Presence of the "A" and "C" peak indicative T/C hetrozygous allele.

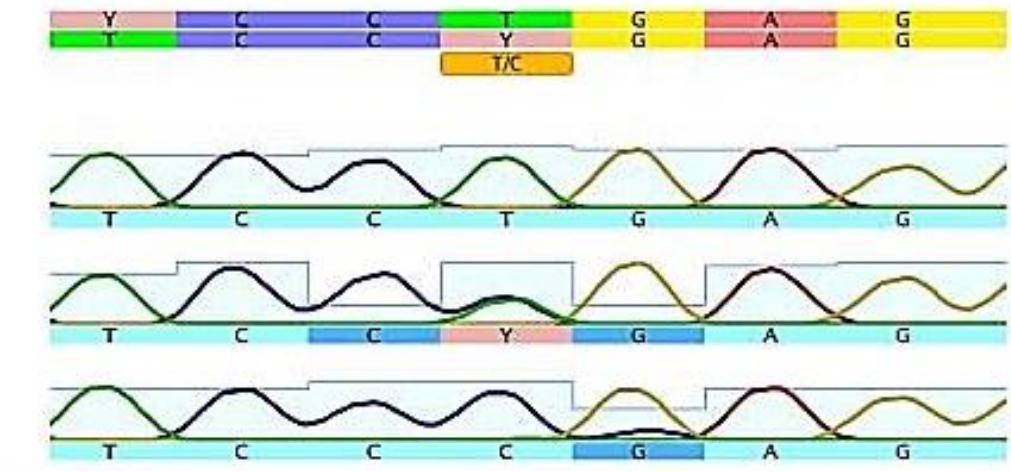
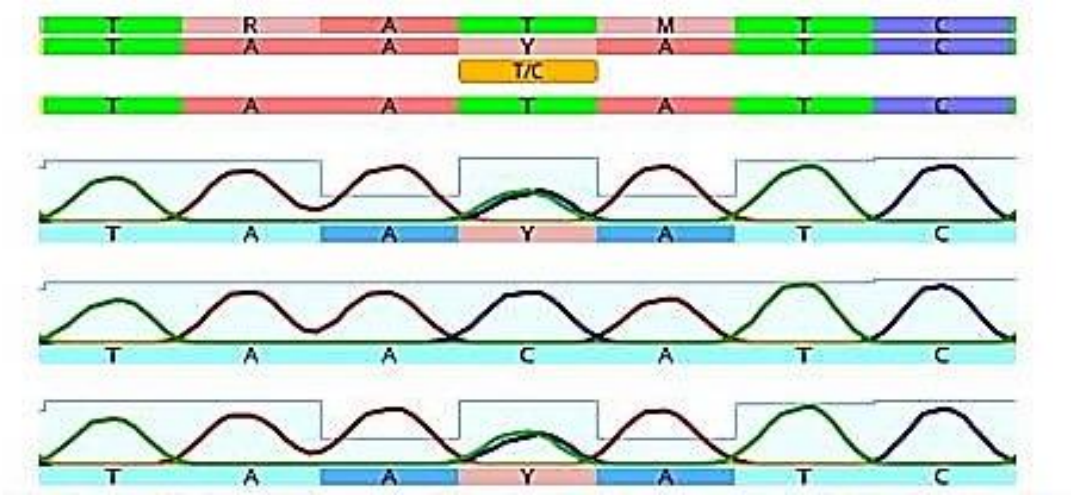


Figure 6. Analysis of SNP rs1800871 of IL-10 gene using sagner sequencing. Single "T" Peak indicative of an T homozygous allele. Sinlge "C" peak indicative of C homozygous allele. Presence of the "T" and "C" peak indicative T/C hetrozygous allele.



Discussion

These findings agreed with (Bakhraibah, et al, 2020) who reported that in comparison with younger/middle aged adult, sensitivity to infections in children <10 years of age was shown to be significantly less, whereas estimated sensitivity for infections in adults >60 years is greater. There is some indication that SARS-CoV-2 may be highly spread in secondary/high schools than primary schools, with a possible effect of class sizes on such spread. It has also been evidenced that spread in schools is more restricted after implementation of some mitigation procedures. Many possible biases which can influence such studies have been discussed [11]. Also Goldstein, et al, [2020] found an indication of significantly lower susceptibility to infections in children < 10 years in comparison with adults who were similarly exposed, for increased susceptibility to infections in adults >60 years in comparison with adults of younger/middle ages, and for risk of SARS-CoV-2 infections related to sleeping close to infected people. Furthermore, published serological researches indicated that young adults (especially < 35y) usually have high cumulative SARS-CoV-2 infection rates in a community [12]. Furthermore, Undurraga, et al, (2021) found the old people with ≥ 80 years have adjusted CFR of 56.82% (95% CrI: 55.25–58.34%) for men and 41.10% (95% CrI: 40.02–42.26%) for women [13]. In addition, the results of prevalence according to gender agreed with [Ahmed, et al, 2021] who found that 56.36% of COVID-19 suspected cases were female. The perceived COVID-19 symptoms showed no significant gender difference in suspected cases while in confirmed cases females [14]. Also Parums, et al,(2023) showed in EG.5 (Eris) infections that females were more than males infected patients [15]. These results mathched with (Asfaram, et al, 2024) who concluded that prior to COVID-19 pandemic, antibodies to *toxoplasma* were discovered in 39% of people (IgG: 38%, IgM: 0.5%, and IgG-IgM: 0.5%). Eleven risk factors were assessed and the result was contact with soils as well as people awareness were highly related to toxoplasmosis. Nevertheless,, factors like females, (20-39) year age group, high schools, housewives, rural area, eating of raw meats or vegetables, washing vegetables or fruits by water, not by detergents, as well as cat owning showed no significant associations with seropositivity. Following the COVID-19 pandemic outbreak, there was an increase in the overall seroprevalence for anti-T. gondii antibodies to 49.7% (IgG: 47.7%, IgM: 0.5%, and IgG and IgM: 1.5%), and 26% of these patients were positive for COVID-19. In addition, prior to COVID-19 pandemic, 40 samples showed negative results for *T. gondii* antibodies, However they became positive later. The adjusted and crude models indicated that toxoplasmosis can represent a potential risk factor for elevated susceptibility to COVID-19 infection, with an odd ratio (OR) of 1.28 (95% confidence interval (CI), 0.82-1.99; P<0.05) [9]. Moreover [Galván-Ramírez, et al, 2023] found that afterward, anti-Toxoplasma IgM and IgG antibodies have been investigated by ELISA. IgM and IgG antibodies to toxoplasmosis showed positive results in 105/384 (27.34%) and

(26/191) 13.6% patients, respectively. In patients whose ages were>40 years, the positivity for both infections was higher. The prevalence rate of S1/S2 SARS-CoV-2 was 308/384 (80.2%), and the Toxoplasma antibody rate was 27.34% among Maxicans [16]. Also [Abdeltawab, et al, 2024] reported that levels of TNF-α were higher in patients with COVID-19 compared to the healthy controls. There was no significant relationship between toxoplasmosis seroprevalence and the existence and severity of COVID-19. In both seronegative and seropositive patients with COVID-19, there was a significant elevation in cytokine levels compared to the controls. Further explorations are needed to be done by mass studies to reveal the high prevalence rate of toxoplasmosis and its correlation with COVID-19 [17]. In addetion [Yudhawati, et al, 2022] demonsetrated mean serum IL-1β levels reduced on day 3 then elevated on day 6. Nevertheless, mean IL-10 elevated on day 3 and on day 6. No significant relationship was found between IL-1β:IL-10 ratio and COVID-19 severity at any time-point. The cutoff values of S. IL-10 between the two groups on days 0, 3, and 6 was 1.09 pg/mL (sensitivity: 66.6%; PPV: 71.4%), 2.11 pg/mL (sensitivity: 67.7%; PPV: 50.0%), and 2.08 pg/mL (sensitivity: 78.6%; PPV: 70.9%), respectively [18]. Furthermore, Carlini, et al, (2023) stated that IL-10 can function as a signal of endogenous danger, secreted by tissues undergoing damages in an attempt for protecting the microorganism from harmful hyperinflammations. The aim of pharmacological plans was potentiating or restoring IL-10 immunomodulatory actions that may represent novel promising avenue for counteracting cytokine storms which arise from hyperinflammations and effectively mitigates serious complications [19]. With co- infection with Toxoplasmosis, in the sequence of TNF, Fricke-Galindo, et al, [2022] stated that the influence of genetic variants in expressing tumor necrosis factor-α and its receptors in coronavirus disease 2019 (COVID-19), severity was not explored before. The associations of TNF (rs1800629 and rs361525), TNFRSF1A (rs767455 and rs1800693) and TNFRSF1B (rs1061622 and rs3397) variants with COVID-19 severity were evaluated as invasive mechanical ventilation (IMV) requirements, and serum levels of soluble TNF-α, TNFR1 and TNFR2 in severe COVID-19 patients were assessed [20]. On the other hand, Carminati, et al, [2022] found a statistically significant variation in the TGF-β1 gene expression between CT and TT genotypes of the rs1800469 polymorphisms, with the less gene expressions in the TT genotype presence. In regard with rs1800468 polymorphisms, a non-significant difference was detected in the expression of TGF-β1 gene in correlation with the investigated genotypes [21]. Also, another study illustrated that IL-1β -511 T > C, and IL-1Ra was associated with COVID-19 severity. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for genotyping of IL-1 gene polymorphism (IL-1+3953 C>T, IL-1β -511 T>C) Hameed, et al, [2025], [22], while the Random Amplification of Polymorphic DNA was used for (RAPD e IL-1Ra genotyping. Sanger sequencing was used for PCR-RFLP data validation. The T allele of the IL-1 + 3953C > T polymorphism frequency was shown to be higher in mild cases in comparison with the severe cases, showed effects against the severity of COVID-19 (P=0.001), Verma, et al, [2024], [23]. Also, the study agreed with [Hammed, et al, 2025] who concluded that an anti-inflammatory cytokine whose levels are elevated in patients with severe COVID-19. IL-10 polymorphisms may play a role in increasing IL-10 levels and the severity of COVID-19. This study aimed to investigate the relationship between IL-10 single nucleotide polymorphisms (SNPs) (rs1800896 [-1082 C < T], rs1800871 [-819 A > G], and rs1800872 [-592 T > G]), [24], Also Hameed et al, [2024] and Nasralla, et al, [2024] reached to similar results [25,26]. Furthermore, these findings agreed with () who rported that the relationship between IL-10 single nucleotide polymorphisms (SNPs) (rs1800896 [-1082 C < T], rs1800871 [-819 A > G], and rs1800872 [-592 T > G]) and the severity of COVID-19 in patients from Kermanshah Province, Iran [27].

Conclusions

The results revealed there were a highly levels of TNF, IL-1ß and IL-10 concentrations with Eris Coved 19 co-infection with Toxoplasmosis. Also, the results found a variation occurence in rs16944 SNP to IL1B GENE ID 3553 that TT was changed to CC in samples number 1,9 and 10. While there was a varation occurence rs1143627 SNP of IL1B GENE ID 3553 changed from CC to TT in samples number 1,9,10 and 13 and variations occurence in rs16944 SNP to IL1B GENE ID 3553 that TT was changed to CC in samples number 1,9 and 10. While there was a varation occurence rs1143627 SNP of IL1B GENE ID 3553 changed

from CC to TT in samples number 1,9,10 and 13. In addition, a variation occured in rs1800871 SNP to IL10 GENE ID 3586 that TT was changed to CC in sample number 1,4,,7, 9,10,11,14 and 15. Also, there was a varation occured in rs1800872 SNP of IL1B GENE ID 3553 that was changed from CC to TT in sample number 1,4,,7, 9,10,11,14 and 15.

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