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Expression of TNF-α miRNA 155 in Eris (EG.5) patients associated with toxoplasmosis

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Abstract

In this case-control study, 100 patients infected with Eris were recruited to Al-Numan Hospital in Baghdad City, and 50 healthy people were also selected as a control group during the period from July 2024 to August 2024.

The results showed that the males who were infected with Eris (EG.5) were higher 52 (52.0 %) than females 48 (48.0%) with non-significant variation (P=0.81). The results also demonstrated there were equal cases of Eris (EG.5) 50 (50 %) recorded in the urban and rural areas with a non-significant difference (P= 0.87). The levels of Toxo-IgM IU/ml were higher among patients infected with Eris (EG.5) (2.78±6.69) than the control group (0.07-0.15), with highly significant variation (p≤0.0001). The levels of Toxo-IgG IU/ml were higher in patients infected with Eris (EG.5) (2.12±3.13) than the control group (0.08 ±0.15) with highly significant variation (P≤0.0001). The levels of IL-1β pg/ml were higher in infected patients (24.21±8.39) than the control group (5.03±2.65) with highly significant variation (P≤0.0001), and the levels of IL-10 pg/ml were higher in infected patients (10.42±2.42) than the control group (4.05±2.02) with a highly significant difference (P ≤0.0001). The levels of TNF-α pg/ml were higher in infected patients (18.58±8.23) than the control group (4.65±1.91) with highly significant variation (P≤0.0001). The Ct value of miR155 of control groups was (18.63±0.17), while the Ct value of patients group was (18.57±0.24), with no significant difference. The computed ratio for gene fold expression was 3.46 for the patients group and 1.46 for the control group.

Keywords: Expression, TNF-α miRNA155, Eris coronavirus, Toxoplasmosis.

Introduction

COVID-19 is brought on by the SARS-CoV-2 coronavirus. It frequently spreads among those who are nearby. The COVID-19 vaccine provides great protection against a deadly illness and death [1]. The newest corona variant (EG.5): The spikes amino acid's profile for EG.5's ancestor, XBB.1.9.2, is the same as that of XBB.1.5. On February 17, 2023, the first case for EG.5 was recorded, and in July 19, 2023, it had been labeled as variant under monitoring (VUM) [2]. Based on this risk evaluation, EG.5 and its sub-lineages are a variant of interest (VOI). When compared to XBB.1.5 and its parent XBB.1.9.2 subvariant, EG.5's spike protein carries an additional F456L mutation [3]. The subvariant EG.5.1 of the EG.5 lineage makes up 88% of sequences available at present for EG.5 with its child lineage and has an additional spike mutation Q52H. 7354 EG.5 sequences from 51 different countries had been submitted to GISAID as of August 7th, 2023 [3]. The majority of EG.5 sequences (30.6%, 2247 sequences) come from China. The other countries with at least 100 sequences include the United States (18.4%, 1356), Republic of Korea (14.1%, 1040), Japan (11.1%, 814), Canada (5.3%), Australia (2.1%, 158), Singapore (2.1%, 154), United Kingdom

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(2.0%, 150), France (1.6%, 119 sequences), and Portugal [4-6]. Across the board, the proportion of reported EG.5 has been rising gradually. 17.4% of people worldwide have EG.5 according to data of epidemiologic weeks 29 (17–23) July, 2023. Four weeks earlier (week 25, 19 to 25 June 2023), there were 7.6% of cases worldwide, which is a considerable increase [5]. Prevalence of EG.5, the latest coronavirus subvariant: Center for Disease Control and Prevention (CDC) determined that EG-5, more than any other single SARS-CoV-2 strain in circulation, was in charge of 20.6% of COVID-19 cases in the United States as of the end of the third week of August [5]. A strain known as FL 1.5.1 (or Fornax), which is supposedly expanding swiftly in the U.S., was in second place the same week and accounted for 13.3% of cases, following a mixture of various XBB strains and children of Omicron [6]. Differences between EG.5 and other recent coronavirus strains: It is not significantly different from other recent strains. The progenitor of the EG.5 variety, identified in February, is the Omicron variant, which first manifested in November 2021 and has undergone multiple subvariants. It might be significant to note that the original strain of Omicron, the SARS-CoV-2 virus, and the earlier, more hazardous Alpha and Delta strains are no longer in circulation, except in exceedingly rare instances [7-9]. However, a unique mutation in the EG.5 spike protein (the element that facilitates virus entry into the host cell) may allow the virus to partially evade the protection produced by infection or vaccination. This variation displays a little bit greater immunological evasiveness than the others due to a little genetic change [10]. The World Health Organization (WHO) has classified EG.5 as a "variant of interest," meaning that states should examine it more closely than other strains because of changes that could make it more contagious or severe. The CDC's page on variant categorization (8) has not yet undergone any revisions [11]. Two genes that encode tyrosine hydroxylase are present in the *T. gondii* genome, which leads to the production of levodopa (L-DOPA), a precursor to dopamines. Tyrosine and phenylalanine are metabolized by encoded enzymes. There will be a constitutive expression of TgAaaH1, which is one of the *Toxoplasma* genes; moreover, bradyzoite formation induces the TgAaaH2 gene during the cyst formation life cycle [12]. Toxoplasmosis is correlated with bipolar disorders and some behavior problems. Toxoplasmosis is primarily related to immunocompromised persons, such as cancer, HIV, and AIDS patients, in addition to transplantation recipients [13]. Many coinfections with toxoplasmosis increase with the high prevalence of infected persons. It is of importance to mention that when such patients are not diagnosed immediately, they may progress to deadly encephalitic toxoplasma infection. A few studies were also performed on patients with toxoplasmosis and cancer, as well as transplantation recipients [14]. Nevertheless, HIV was shown to be a comorbid disease in 40% of toxoplasmosis patients and accounted for more than 50% of direct healthcare costs related to clinical toxoplasma infection [15]. miR-155 induction was driven by TNF-α, whose effect was considerably boosted by IFN-γ. Two functional miR-155-binding sites are present in the PD-L1 3'-UTR [16]. The kinetics and maximal level of PD-L1 induction on TNF-α IFN-γ treatment is controlled by the endogenous miR-155. Studies obtained similar results in dermal fibroblasts, suggesting that the pathways of IFN-γ/TNF-α/miR-155/PD-L1 are not confined to HDLEC. Those findings indicated that miR-155 is a critical constituent of the inflammation-induced regulatory loops that control PD-L1 expressions in the primary cells [17].

Materials and methods:

In this study, 100 patients (52 males and 48 females) infected with Eris (EG.5) Virus were recruited in Al-Numan Hospital in Baghdad City, and 50 healthy persons (25 males and 25 females) were also selected as a control group during the period from July 2024 to August 2024.

The age of patients and controls ranged from 11 to >55 years. The study focused on people who had

symptoms of the coronavirus and were then confirmed to have the Eris (EG.5) variant.

From each participant (6) ml of blood sample was collected and divided into two parts: the first part was put in EDTA tubes and the second part was put in gel tubes and left at room temperature for about 15– 30 minutes to clot, then centrifuged at 3000 rpm for 5 minutes to get serum. The 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, we analyzed the levels and gene expression levels of miRNA and TNF-α using RT-qPCR techniques. The correlation between these molecular markers and the clinical status of the Eris (EG.5) variant, along with the presence or absence of toxoplasmosis co-infection, formed the core analysis of the study.

Gene Expression one step for TNF-α (mRNA):

Analysis and calculation of gene expression levels of one or more genes depended on RNA concentration after conversion to cDNA. All processes included total RNA purification, qPCR amplification, and data analysis.

Table (1): Primer Name, Sequence, and Annealing Temperature (°C)

Primer Name	Sequence	Annealing Temp. (°C)
β-Globin-F	ACACAACTGTGTTCACTAGC	65
β-Globin-R	CAACTTCATCCACGTTCACC	
TNFa_exp-F	CTCTTCTGCCTGCTGCACTTTG	60
TNFa_exp-R	ATGGGCTACAGGCTTGTCACTC	

Statistical Analysis:

Analysis of data was carried out using the available statistical package of IBM SPSS-22 (IBM Statistical Packages for Social Sciences- version 22, Chicago, IL, USA). Data of the current study were analyzed by Chi-square.

Results:

The results of the current study observed the mean age of cases was (37.49±16.19) years versus (41.10±15.94) years for the control group with non-significant variation (P= 0.19). The findings also demonstrated that the age groups in the second to fourth decade of age (26-40) had 36 (36%) cases of Eris (EG.5) out of 100 higher than other age groups, followed by the age (11-25) and (41-55) years which were 26 (26%) and 22 (22%) respectively. Fewer cases of Eris (EG.5) were detected 16 (16%) in the age groups >55, compared to control groups. These differences were statistically non-significant (P = 0.58) as demonstrated in table (2). The results showed that the males who were infected with Eris (EG.5) were higher 52 (52%) than females 48 (48%) with non-significant variation (P=0.81). The result also demonstrated there were equal cases of Eris (EG.5) 50 (50%) recorded in the urban and rural areas with a non-significant difference (P= 0.87), as illustrated in table (2).

The levels of Toxo-IgM IU/ml were higher among patients infected with Eris (EG.5) (2.78±6.69) than the control group (0.07-0.15), with highly significant variation (p≤0.0001). The levels of Toxo-IgG IU/ml were higher in patients infected with Eris (EG.5) (2.12±3.13) than the control group (0.08 ±0.15) with highly significant variation (P≤0.0001). The results also revealed that the levels of Eris -IgM IU/ml were higher in infected patients than the controls (1.9 ±-1.04 and 0.08±-0.15), respectively, with highly significant variation (P≤0.0001). Also, the levels of Eris -IgG IU/ml were higher in infected patients than the controls 14.32±6.76, and 0.09±0.162 respectively, with highly significant variation (P ≤0.0001).

The levels of IL-1β pg/ml were higher in infected patients (24.21±8.39) than the control group (5.03±2.65) with highly significant variation (P≤0.0001), and the levels of IL-10 pg/ml were higher in infected patients (10.42±2.42) than the control group (4.05±2.02) with a highly significant difference (P ≤0.0001). The levels of TNF-α pg/ml were higher in infected patients (18.58±8.23) than the control group (4.65±1.91) with highly significant variation (P≤0.0001).

Results of the current study revealed that the levels of TNF-α pg/ml were higher in infected patients than the controls (18.58±8.23 and 4.65±1.91), respectively, with a highly significant difference (P≤0.0001) as shown in table (3).

Gene expression was measured for the miR155 gene in the present study, a fluorescent dye that recognizes any double-strand DNA including cDNA, the amplification was recorded as a Ct value (cycle threshold). The lower Ct value indicated the presence of higher copies of the target and vice versa. In this study, we analyzed the gene expression for the miR155 gene analyze and estimate by RT-PCR depending on Ct value in both groups Toxoplasmosis patients and in apparently healthy individuals.

The Ct value of the RNU43 housekeeping gene used in the present study is shown in table (4). The Ct value was (27.71±1.52) in the controls, while the Ct value was (28.02±2.45) in the patients' groups. Non-significant variations were observed between those groups regarding the mean Ct value of RNU43, suggesting that the internal gene may be expressed stably in both tissues and cells in both groups for the experiment.

The Ct levels were inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the Ct level, the greater the amount of target nucleic acid in the sample).

The Ct value of miR155 of the control group was (18.63±0.17), while the Ct value of the patients group was (18.57±0.24), with no significant difference between these groups.

The results in table (4) also showed that the mean $2^{-\Delta\Delta Ct}$ to MicroRNA155 of the controls was (0.00±1.60), while it was (-0.37±2.38) of patients group. The computed ratio of the gene fold's expression was 3.46 for the patients and 1.46 for the controls.

Ct values of B.Globin housekeeping genes used for TNF-α MicroRNA was showed in the table (5). İn the control group, Ct value was (14.71±1.93), while the Ct value was (14.04±3.05) in the patients' group. Non-significant variation was detected between the groups regarding mean Ct values of B.Globin.

Table (2): Demographic picture of the study groups

Variable		Groups		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 (26%)	8 (16%)	0.58
	(26-40)	36 (36%)	20 (40%)	
	(41-55)	22 (22%)	13 (26%)	
	>55	16 (16%)	9 (18%)	
Total		100 (100%)	50 (100%)	
Gender	Male	52 (52%)	25 (50%)	0.81
	Female	48 (48%)	25 (50%)	
Total		100 (100%)	50 (100%)	
Residency	Urban	50 (50%)	26 (52%)	0.87
	Rural	50 (50%)	24 (48%)	
Total		100 (100%)	50 (100%)	

Table (3): The mean levels of immunological parameters between cases (n=100) and control (n=50)

	Groups	Mean±SD	SE	Max-Min	T-test	P-value
Toxo-IgM (IU/ml)	Case	2.78±6.69	0.66	.16-31.95	4.03	≤0.0001
	Control	0.07-0.15	0.021	.01-0.66		
Toxo-IgG (IU/ml)	Case	2.12±3.13	0.31	0.15-14.45	6.53	≤0.0001
	Control	0.08 ±0.15	0.022	.01-0.66		
Eris-IgM (IU/ml)	Case	1.9 ±-1.04	0.10	1.00-6.50	17.36	≤0.0001
	Control	0.08±-0.15	0.02	0.01-6.6		
Eris-IgG (IU/ml)	Case	14.32±6.76	0.67	0.01-33.30	21.09	≤0.0001
	Control	0.09±0.162	0.02	0.01-0.66		
IL-1β (Pg./ml)	Case	24.21±8.39	0.83	10.72 -48.30	20.85	≤0.0001
	Control	5.03±2.65	0.37	1.09 -18.90		
IL-10 (Pg. /ml)	Case	10.42±2.42	0.24	4.53-16.27	16.96	≤0.0001
	Control	4.05±2.02	0.28	1.67-12.98		
TNF-α (Pg./ml)	Case	18.58±8.23	0.82	1.26-46.55	16.06	≤0.0001
	Control	4.65±1.91	0.27	1.09-9.43		

Table (4): Comparison between the study groups in CT value of RNU43 and miR155 expression

Groups	RNU43 (M±Std)	miR155 (M±Std)	miR155 (M±Std)	miR155 (M±Std)	Folding
	CT	CT	DCT	DDCT	
Case	28.02±2.45	18.57±0.24	-9.45±2.38	-.37±2.38	3.46
Control	27.71±1.52	18.63±0.17	-9.08±1.60	0.00±1.60	1.46
T-test	0.30	0.53	0.356	0.357	1.405
P-value	0.76	0.60	0.728	0.727	0.86

Table (5): Comparison between different groups in CT value of B.Globin and TNF-α expression

Groups	B.Globin (M±Std)	TNF-α (M±Std)	TNF-α (M±Std)	TNF-α (M±Std)	Folding
	CT	CT	DCT	DDCT	
Case	14.04±3.05	28.66±1.41	14.62±1.76	-1.09±1.76	5.60
Control	14.71±1.93	30.43±1.21	15.72±1.28	0.00±1.28	1.37
T-test	0.52	2.50	1.36	1.36	1.12
P-value	0.61	0.03	0.20	0.20	0.28

Discussion:

Eris (EG.5) Coronavirus is a new mutant that has caused many infections in Iraqi cities, including Baghdad, but it is less dangerous than the original COVID-19. According to the results, the males who were infected with Eris (EG.5) were higher than females. This result agreed with Parums (2023), who reported that males were more susceptible to Eris (EG.5) due to their work and contact with society [18]. Levels of Toxo-IgM IU/ml were higher among patients infected with Eris (EG.5) than the control group. Abdeltawab, et al, (2024) proved that the infection rate with *Toxoplasma gondii* was 25% among Covid-19 infections, These commonly detected infections are attributed to the fact that Toxoplasma is a parasite that lives intracellularly in the body's organs and that its spread is due to weak immunity, which confirms that the coronavirus weakens the body's immune system, making it vulnerable to side effects [19].

The levels of IL-1β pg/ml were higher among infected patients with Eris (EG.5) than the control group, and these findings were in harmony with (Fawzy et al., 2022), who stated that IL-1β is a pro-inflammatory cytokine that has been implicated in pain, inflammation, and autoimmune conditions. This review focuses on the roles of the intracellular complex, the inflammasome, which regulates IL-1β production as well as their roles in infection with the Eris coronavirus [20].

The levels of IL-10 were higher among infected patients than the control group. Islam, et al, (2021) reported that IL-10 is a cytokine with potent anti-inflammatory properties that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis this is why it appears in high concentrations in Covid-19 infections, depending on the severity of the infection [21].

The levels of TNF-α were higher among infected patients than the control group, and these current results were in line with Mortaz et al. (2021), who showed that TNF-α is a key regulator of acute and chronic inflammation and, in certain circumstances, causes cell death by apoptosis and necroptosis. TNF-α mediated inflammations may result in the damage of detrimental tissues and is gradually developed to pulmonary fibrosis, which then leads to pneumonia, lung oedema as well as acute respiratory distress syndrome in addition to their folding expressions (Hameed et al., 2025), [22-25]. Tumor necrosis factor may be increased when there is a co-infection between Toxoplasma and Eris Corona virus. The mean $2^{-\Delta\Delta Ct}$ to MicroRNA155 of the control group was (0.00±1.60), while it was (-0.37±2.38) in the patients group. Computed ratios of the gene fold's expression were 3.46 for the patients group and 1.46 for the control group. The gene expression for the B.Globin gene was analyzed and estimated by RT-PCR depending on Ct value in both toxoplasmosis patients and in apparently healthy individual groups [23].

Ct values of B.Globin housekeeping genes were used for TNF-α MicroRNA, showing that Ct value was (14.71±1.93) in the controls, while Ct value was (14.04±3.05) in the patients group, with no significant difference regarding the mean Ct value of B.Globin. In the assay of real-time PCR, positive reactions were observed by fluorescent signal accumulation [26]. Ct (cycle threshold) is known as the cycle numbers needed for the fluorescent signal for crossing a threshold (i.e., to exceed background levels). Anari et al., (2023) reported that MicroRNA-155 (miR-155) plays a role in the post-transcriptional gene regulation of hematopoiesis, oncogenesis, and inflammation [27]. A Ct level is inversely proportional to the target nucleic acid's amount in a sample (i.e., the lower the Ct levels, the greater the f target nucleic acid amount in a sample), which agreed with Jihad and Issa (2021). Moura et al., (2019) concluded that microRNA-155 inhibits fibroblast growth factor 7 expression in diabetic skin and decreases wound inflammation [28,29]. Also, Nasralla et al. (2024) reported that TNF-α has folding expressions [30].

Conclusions:

Based on the results, it was found that the microRNA-155 for tumor necrosis factor recorded a significant increase compared to the control group, which proves the influence of the pathological condition of Covid-19 Eris co-infection with toxoplasmosis.

Conflict of interest: NIL

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