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Correlation between Interleukin-6, Toxoplasmosis, and Cytomegalovirus in Novel COVID (nCoV-19) patients in Kirkuk–Iraq

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Abstract

Background*, microbes like Cytomegalovirus (CMV) and Toxoplasma gondii (T. gondii) have significantly influenced public health, especially in women, resulting in birth anomalies that were too frequent in Kirkuk city. Patients infected with SARS-CoV-19 are more likely to develop this illness. This research aimed to examine the association between COVID- 19, CMV, and T.gondi and to evaluate changes in the patient's level of Interleukin-6 (IL-6).* ***Setting:*** *The observational cross-section study was applied to two groups, study group 162 with positive for 2nCovid-19 and control 50 individuals with negative for novel Covid.* ***Methodology:*** *ELISA kits were set for assessing T.gondii and CMV, both IgG and IgM antibodies, while a cassette test (ELISA+ fluorescence) was applied for detecting IL-6.* ***Results:*** *The overall rate of 76.74% for CMV was higher than 45.93% for T.gondii. Immunoglobulin M rates were 16.27 % for T.gondii versus 20.34 for CMV. Meanwhile, T.gondii IgG was 29.65 % in contrast to 56.39 % for CMV, P<0.05. The relationship was significant according to age, sex of individuals, and the incidence of T.gondii, CMV. The mean value of IL-6 in females was 38.98 Pg/ml versus 37.10 Pg/mL in sera of males compared to control below 7 Pg/ml. The high mean level of IL-6 was 80.6 in females and 70.57 Pg./mL in males, recorded among patients aged 51 to 60 compared to 8.66 Pg/mL in patients aged 10 to 20 years, P<0.05. A positive correlation was seen between Toxo IgM and CMV IgM versus a negative correlation between IgG antibodies. The positive link is exerted between IL-6 and CMV IgM only.* ***Conclusions:*** *the rates of T.gondii and CMV among patients positive for Covid 19 were high. Co-infection of COVID and CMV in females and the elderly had a substantial impact on the immune system, in particular, IL-6.*

Keywords: *Covid, CMV, Toxoplasma, ELISA, Interleukin-6.*

INTRODUCTION

The coronavirus is a member of the Coronavirinae family of viruses, found in the Nidovirales order. Severe Acute Respiratory Syndrome (SARS), newly identified as nCoV-19, may be caused by the Ortho-coronavirinae subfamily, which includes the causal agent [1]. The four genera in the family are alpha-coronavirus, beta-coronavirus, gammas and deltas. On the other hand, alpha-coronaviruses do not have an extensive enough host range to encompass avian species. The Beta-CoVs. COVID- 19, MERS-CoV, and SARS-CoV were among the most lethal human viruses listed. They threaten public health, veterinary care, and the economy because they infect people, other mammals, bird species, cattle, and companion animals. The virus may spread during

outbreaks like MERS, SARS, and COVID-19 since animal Covid·.s are seldom transferred to humans. Beta-coronaviruses can infect only animals. Coronavirus infections in people and animals cause enteritis and respiratory disorders [2, 3]. A pandemic of pneumonia with an unknown origin was reported to WHO in the Kurdistan region of Iraq in mid-February 2020, following the initial case in Wuhan, China, on December 19, 2019. China's Hubei Province China's government has designated 2019- nCoV.

Thousands of human ailments have been linked to this virus in various parts of Iraq. Infections with no symptoms, illnesses with mild symptoms, conditions with severe symptoms, and even deaths have been reported [4]. Because Kirkuk Province is a crossroads for Northern Baghdad, Tikrit, and Diyala, additional reported cases were registered in Kirkuk after February 2020.

In response to tissue damage and infections, the cytokine IL-6 is produced [5]. Several cell types, including fibroblast, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells, and T and B cells, manufacture this cytokine [6]. After binding to its specific receptor, IL-6 starts a chain of signaling events primarily related to the JAK/STAT3 activation pathway [7]. These events promote the transcription of many downstream genes involved in cellular signaling processes, such as cytokines, receptors, adaptor proteins, and protein kinases. IL-6 modulates the host immune response and promotes viral infection. IL-6, along with IL-1 and TNF-a, is a key cytokine during infection [8]. Experiments using IL-6-deficient animals confirm IL- 6's relevance during viral infections. Using this model, [9, and 10] found that IL-6 promotes optimal T-cell response regulation, inflammation resolution, tissue remodeling that helps the lungs heal, migration and phagocytic activities of macrophages, and regulation of IgG isotope switching.

Toxoplasma gondii is an intracellular apicomplexan protozoan parasite that inhabits birds and animals, including humans, as intermediate hosts. Still, its reproductive phase can only be completed in definitive feline hosts, most often domestic cats. It is mainly spread in human society by semi-domestic-domestic animals. Semi-feral cats feed on infected rodents and birds and then consume them. Consequently, the infectious oocysts are discharged via their feces and may cause illnesses in people through direct contact [11]. Alternately, these oocysts may infect domestic animals, and eating their contaminated flesh would spread *T. gondii* to humans. Clinical symptoms of acquired Toxoplasmosis often resolve on their own in immunocompetent persons, but immune- deficient patients have an increased risk of developing an opportunistic infection. The second image is the incidence of congenital anomalies such as stillbirth, encephalitis, microcephaly, and abortions, predominantly in married women [12]. The association or link between Toxoplasmosis and viral infection is not evident because the Dense Granule Protein-7 (GRA-7) is secreted into the host cell by *Toxoplasma gondii* and suppresses viral replication. More incidences of congenital abnormalities and abortions prompted increased focus on Toxoplasmosis in Kirkuk Province after 1992[13]. From 1992 to 2012, a serological test using ELISA in Kirkuk City ELISA performed; the study revealed the seropositivity rate varied from 10 to 55.6 %.

Cytomegalovirus is the most common congenital disability in the world. It is common in the U.K. and affects about three out of every thousand newborns [14]. In Iraq, the infection rate was high, but no estimates were available, especially for Kirkuk [15]. It could cause nerve damage, like going deaf [16]. CMV in prenatal transmission can happen when a mother gets infected during the third trimester of gestation. It can also happen to women who are immune to the virus if it returns or receives a new strain [17]. But most babies don't have any symptoms, especially if they were born early. CMV is easily transmitted from mother to child through breastfeeding and other close contacts. CMV is often passed from one child to another in daycare and similar settings in wealthier countries like the U.K., where breastfeeding is less common and lasts less time than in developing countries. Most young children with CMV infection don't have any flu-like symptoms. People who are not infected but are close to young children are at risk of getting CMV.

This is especially scary for female childcare workers of childbearing age[18]. Transmission between married couples is quite prevalent. CMV incidence varies globally [19]. In Japan, the primary rate of CMV-IgG antibodies among 18-year-old women declined from 93.2% to 66.7% from 1980 to 1998 using complement fixation test; they found that age and parity of pregnant women were connected with immunological status; 35.6% of recent young premature were vulnerable [20]. CMV- IgM was found in 6% of Palestinian women's sera [21]. Turkish women had 94.9 percent CMV-IgG antibodies and 0.4 percent CMV-IgM antibodies [22]. In the same country, 97% of women had CMV-positive sera, while only 1% had primary CMV-IgM25. Only 37 of 572 Irish women tested had CMV-IgM antibodies [23], which was conducted over three years [24]. ELISA testing on 343 women with Bad Obstetric History in Kirkuk Province, Iraq, indicated that 24.35 percent of CMV sera were positive, with CMV- IgM accounting for 17.6 percent and CMV-IgG accounting for 6.75 percent of the positive sera [25]. In the same city, a CMV rate of 49.87% was recorded, and CMV IgG contributed 36.23 % [26]. Toxoplasmosis rates spiked after the 2003 Iraq war[27]. During this time, we focused our attention on the effect of polymicrobial (*Toxoplasma*, CMV), and the early case of SARS2nCovid-19 in Kirkuk city was towering proof. Based on the available information, CMV co-infection with SARS-Covid-19 was not investigated. Therefore, the current study was planned and aimed to prove this correlation.

MATERIALS AND METHODS:

Study information: A mixed cross-sectional and observational study was carried on in Ibn-Alnafees private lab using ELISA for detecting Toxoplasma and Cytomegalovirus and a device (ELISA+ fluorescent) for detecting the level of IL-6 in the serum of 162 patients positive for nCovid-19 using RT-PCR (Case detection in Public Health Laboratories in Kirkuk city) and some private labs) from 81 males and the same number of females. The second group involved 56 individuals negative for COVID-19, and 30 were female with previous birth anomalies. A special form for each patient was filled with complete information, and then the consent and patient permission were taken to participate in the study.

Sample collection and serum extraction:

Under a sterile state, five milliliters of venous blood were taken from each participant, discharged into a jell activator test tube for good clotting, and left for 15 minutes in the water bath at 37^0 C. The sera were centrifuged for 5 minutes using 4000 rpm. Clear, non-hemolysis, non-jaundice, and non-lipemic sera were isolated and transferred into a clean Eppendorf tube, labeled, and kept in a freezer at -20^0 C till use.

Serological methods:

Determination of IL-6: (Immunofluorescent Assay).

The immunofluorescent kit was Purchased from China (Cusabio company) by the means of BIOZEK company-Netherland. Briefly, the test uses anti-human IL_6 monoclonal antibody I conjugated with fluorescence latex coated on the junction of nitrocellulose membrane, sample pad, and another anti-human IL-6 II coated on the test line. After adding 100µl of serum to the sample insertion hole. The fluorescent latex- labeled anti-human IL-6 antibody I binds to the IL-6 in the sample and forms a marked antigen-antibody complex. The latter will move to the test detection zone by capillary action. The notable antigen-antibody complex is captured on the test line by anti-human IL-6 antibody II. The fluorescent intensity of the test increases in proportion to the amount of IL-6 in the sample. This process will be accomplished within 15 minutes. Then the cassette or the device is inserted into (D.C.R. 1000) qualitative-automated analyzer (BIOZEK company-Netherland). The concentration of IL-6 in a sample will be measured and displayed on the screen. The normal range is up to 7 Pg/mL.

***Toxoplasma* IgM and IgG antibodies assessment using ELISA:**

Biozek Company in the Netherlands purchased this ELISA kit from D.R.G. Company in Germany and sent it to our location. Immunoglobulins and subsequent identification of the *Toxoplasma* IgM test based on the ELISA

method include the ELISA kit for *T. gondii*. which is designed to precisely bind an antigen conjugated to peroxidase. The capture approach involves the attachment of solid-phase antigens to monoclonal antibodies on a microplate. The antigen is an active, pure *Toxoplasma gondii* antigen protein. Meanwhile Toxo-IgG Kit was from the same company; the kit was identical to the IgM kit except for the HRP-conjugated solid face antigen on the ELISA strips. The testing procedure and findings were identical to those for IgM. These processes were performed following the manufacturing company and with [28].

Cytomegalovirus IgM and IgG antibodies detection:

The diagnostic serological kits capture ELISA Diagnostic reagent group (D.R.G.)- German company used. Briefly, the idea behind the tests is started when the suspected serum is added to ELISA well; the antibodies bind to solid phase immobilized anti- CMV-IgM, and this reaction achieved 30 minutes to form an immune complex of antigen (Ag) and antibody(Ab). Ag-Ab complex will remain in the well, and after five times of washing, the non-specific bound antibodies will be removed through the washing process. Subsequently, 100 µl of H.R.P. conjugate was added, which led to the strength of Ag-Ab within 30 minutes. The second wash with a working wash solution will remove the residue of nonbound antibodies or any contaminants. After drying the wells, the 100 µl of a substrate(T.M.B) will become the area where the conjugate will work within 30 minutes. Later, the reaction stopped by adding 90 µl of a stop solution, and the intensity of the yellow color increases with the existence of the CMV IgM antibody that is to be fixed by an ELISA reader machine using 450-630 nm wavelength. This procedure was performed and extracted from the leaflet of the kit. On the other hand, CMV-IgG determining was the same as CMV IgM, except the solid phase was specifically labeled by CMV IgG and the type of Conjugate was H.R.P. IgG.

The following are the Toxoplasma and CMV IgM and IgG levels.

Below 9 I.U./mL, the test is negative From 0.9 to 0.99 equivocal result.

Above 11 I.U./mL, the test is positive.

Guidelines for interpreting correlation coefficient r : $0.7 < |r| \leqq 1$ strong correlation

$0.4 < |r| < 0.7$ moderate correlation $0.2 < |r| < 0.4$ weak correlation

$0.0 \leqq |r| < 0.2$ no correlation (Internet source)

Statistical Analysis:

The following statistical equations were used: Fisher test, t-student, and Chi-square test. The mean and standard error of variables in the test and control groups were also calculated. As well as to extraction of negative and positive correlations. All these statistical processes were done after the arrangement of all data in Excel files using a computer statistic program SPSS version 19.

RESULTS:

The total prevalence of *T.gondii* was 45.93 percent, with IgM prevalence at 16.27 percent and IgG prevalence at 29.65 percent. In comparison, the CMV rate was 76.74 percent, with IgM antibodies contributing 20.34 percent and IgG antibodies providing 56.39 percent. Subacute case rates (IgM + IgG) for T.gondii and CMV were 2.32 percent and 6.97 percent, respectively. Suspicious or doubtful positive rates were 13.95 % for T.gondii versus 2.90 % for CMV.Table -1.

Table 1 Percentages of patients with positive and negative tests for *T.gondii* and *CMV.*

Type of antibodies	**T.gondii Positive**		**T.gondii Negative**		**CMV Positive**		**CMV Negative**	
	No.	%	No.	%	No.	%	No.	%
IgM	28	16.27	144	83.73	35	20.34	137	79.66
IgG	51	29.65	70.35		97	56.39	75	43.61

Ig M+G	4	2.32	168	87.68	12	6.97	160	93.03
IgM+IgG equivocal *	24	13.95	148	86.04	5	2.90	167	87.10

***Equivocal means the level was between 0.9 to 0.99 IU/mL. excluded.**

Toxo IgG exhibited higher levels (M = 1.98, SD = 0.65) than Toxo IgM (M = 0.48, SD = 0.06). An independent sample t-test indicated a statistically significant difference, p=0.05, with a one- tailed p-value of 0.0341 (t(5)=-2.31). Table-2.

Table-2. Frequency of *Toxoplasma gondii* IgM and IgG antibodies among females positive for SARS 2nCovid-19

Age group/years Female	**Mean± Standard error**		
	Age	**Toxo IgM**	**Toxo IgG**
10--20	17.82 ±0.6	0.51 ± 0.06	3.07 ± 0.9
21--30	25.48 ± 0.54	0.52 ± 0.03	2.45 ± 0.48
31--40	35.20 ± 0.5	0.52 ± 0.02	3.84 ± 0.62
41-50	45.50 ± 1.37	0.45 ± 0.01	2.50 ± 0.57
51-60	56.30 ± 0.95	0.38 ± 0.04	0.00 ± 0
over 60	68.86 ± 3.18	0.52 ± 0.05	0.00 ± 0
Total	41.53 ± 7.8	0.48 ± 0.06	1.98 ± 0.65

The CMV IgM group (M = 0.85, SD = 0.5) had lower values than the CMV IgG group (M = 1.17, SD = 0.58). A t-test for dependent samples (t(5) = -2.69, for two-tailed p =.043, 95 percent confidence interval [-0.64, -0.01]) showed that this difference was statistically significant.Table-3.

The average level of IL-6 in the blood of women who had Toxo-and CMV IgG antibodies about SARS 2nCovid-19 was 38.69 Pg/ml, which was higher than the normal range of up to 7 Pg/ml. Based on their ages, women aged 51 to 60 had a high level of IL-6 (80.6 27.2 Pg/ml) compared to women over 60, who had a level of 11.7 Pg/ml (p0.05). Table-4.

The Toxo IgM group had lower values (M = 0.66, SD = 0.16) than the Toxo IgG group (M = 1.64, SD = 0.61). A t-test for dependent samples showed this difference was statistically significant, t(5) = -3.67, p = .014, 95% Konfidenzintervall [-1.66, -0.29]. Table -5.

Table 6 shows the average levels of IL-6 based on a man's age. Between 10 and 20 years old, the intermediate grade was 8.66 Pg/mL, while between 51 and 60 years old, it was 70.57 Pg/mL (P<0.05). The average amount of IL-6 in men was 37.10 Pg/mL, while the average amount in women was 38.69 Pg/mL (P>0.05)

Table-3. Distribution of *Cytomegalovirus* IgM and IgG antibodies in serum of women positive for SARS-2CoV-19

Age group/years Female	**Mean ±Standard error**		
	Age	**CMV IgM**	**CMV IgG**
10--20	17.82 ±0.6	1.54 ± 0.08	1.52 ± 0.15
21--30	25.48 ± 0.54	1.02 ± 0.08	1.39 ± 0.09
31--40	35.20 ± 0.5	0.98 ± 0.11	1.31 ± 0.09
41-50	45.50 ± 1.37	0.81 ± 0.06	1.54 ± 0.34
51-60	56.30 ± 0.95	0.73 ± 0.05	1.28 ± 0.11
over 60	68.86 ± 3.18	0 ± 0	0 ± 0
Total	41.53 ± 7.8	0.85 ± 0.2	1.17 ± 0.14

P-value @ 0.05 = 0.043 Moderate significant

The CMV IgM group had lower values (M = 0.95, SD = 0.42) than the CMV IgG group (M = 1.19, SD = 0.19). A t-test for dependent samples showed this difference was not statistically significant, t(5) = -1.13, p = .31, 95% Konfidenzintervall [-0.77, 0.3].

Table 4: Interleukin-6 levels among females with Toxoplasmosis and Cytomegalovirus who were positive for SARS-2CoV-19.

Age group/year Females	**Mean± Stander error**	
	Age	**IL-6 Pg/ml**
10--20	17.82 ±0.6	30.8 ± 8.7
21--30	25.48 ± 0.54	40.7 ± 9.4
31--40	35.20 ± 0.5	29.7 ± 6.8
41-50	45.50 ± 1.37	38.4 ± 18.9
51-60	56.30 ± 0.95	80.6 ± 27.2
over 60	68.86 ± 3.18	11.7 ± 2.02
Total	41.53 ± 7.8	38.69 ± 9.3

Table-5. Prevalence of *Toxoplasma gondii* IgM and IgG antibodies among Males positive for SARS-2CoV-19

Age group/years Males	**Mean±Stander error**		
	Age	**Toxo IgM**	**Toxo IgG**
10--20	16.2 ±1.6	0.8 ± 0.06	1.094 ± 0.84
21--30	27.14 ± 0.6	0.59 ± 0.07	2.07 ± 0.42
31--40	35.77 ± 0.66	0.88 ± 0.219	2.102 ± 0.36
41-50	43.7 ± 0.59	0.47 ± 0.03	2.37 ± 0.48
51-60	55.08 ± 0.59	0.65 ± 0.073	1.17 ± 0.16
over 60	66.6 ± 1.48	0.56 ± 0.06	1.02 ± 0.30
Total	40.75 ± 7.5	0.66 ± 0.16	1.64 ± 0.61
P-value @ 0.05 = 0.034 Moderate significant			

Table-6. Distribution of *Cytomegalovirus* IgM and IgG antibodies in serum of males positive for SARS-2 CoV-19

Age group/years Males	**Mean, Stander error**		
	Age	**CMV IgM**	**CMV IgG**
10--20	16.2 ±1.6	0.84± 0.17	1.03 ± 0.08
21--30	27.14 ± 0.6	0.78 ± 0.033	1.05 ± 0.064
31--40	35.77 ± 0.66	0.94 ± 0.064	1.19 ± 0.071
41-50	43.7 ± 0.59	0.83 ± 0.06	1.53 ± 0.13
51-60	55.08 ± 0.59	0.714 ± 0.037	1.26 ± 0.054
over 60	66.6 ± 1.48	0.66 ± 0.03	1.07 ± 0.087
Total	40.75 ± 7.5	0.95 ± 0.42	1.19 ± 0.19
P-value @ 0.05 = 0.043 Moderate significant			

Table-7. Interleukin -6 levels in males positive for SARS 2nCovid-19 suffering from Toxoplasmosis and Cytomegalovirus. for SARS-2CoV-19.

Age group/year Male	**Mean, Stander error**	
	Age	**IL-6**

10--20	16.2 ±1.6	45.45± 8.8
21--30	27.14 ± 0.6	21.36 ± 3.6
31--40	35.77 ± 0.66	49.09 ± 16.6
41-50	43.7 ± 0.59	22.6 ± 6.3
51-60	55.08 ± 0.59	70.57 ± 19.45
over 60	66.6 ± 1.48	13.53 ± 2.08
Total	40.75 ± 7.5	37.10 ± 7.81

The following figures demonstrate the correlation between *Toxoplasma IgM and CMV IgM*, a positive correlation with r= 0.49, considered a moderate correlation figure-1. Whereas figure- 2 was between Toxo-IgG and CMV IgG revealing a negative correlation as r=0.53. While in Figure 3, the correlation coefficient (r) =0.48 is a positive correlation between IL-6 and CMV IgG antibodies. Moreover, the other parameters' correlation was insignificant, P>0.05.

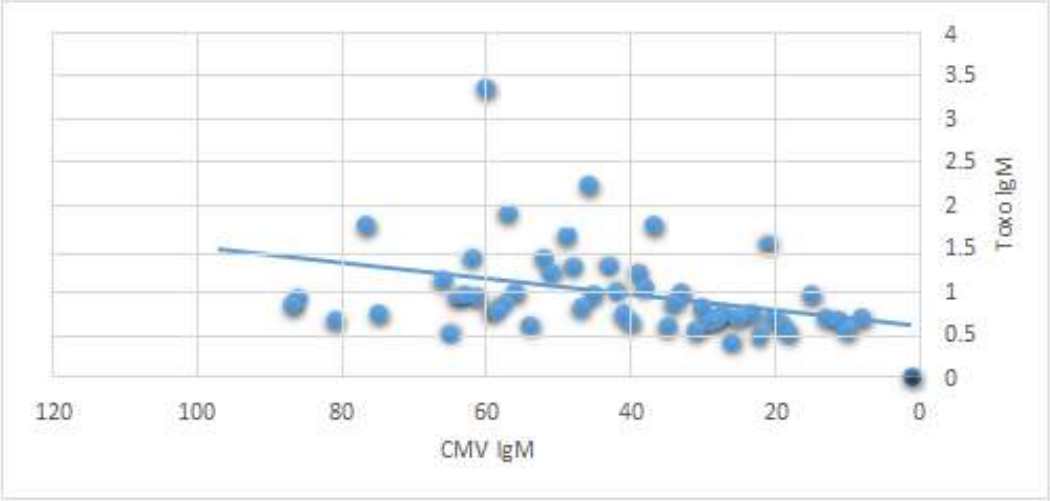

Figure-1. The cross plot between Toxoplasma-IgM and CMV IgM antibody.

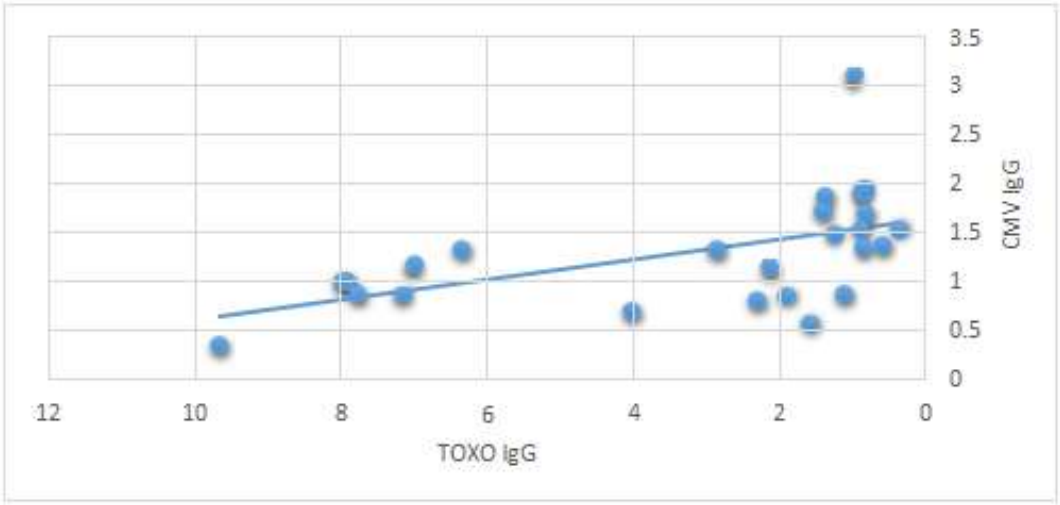

Figure-2. The cross plot between CMV IgG antibody and Toxoplasma-IgG.

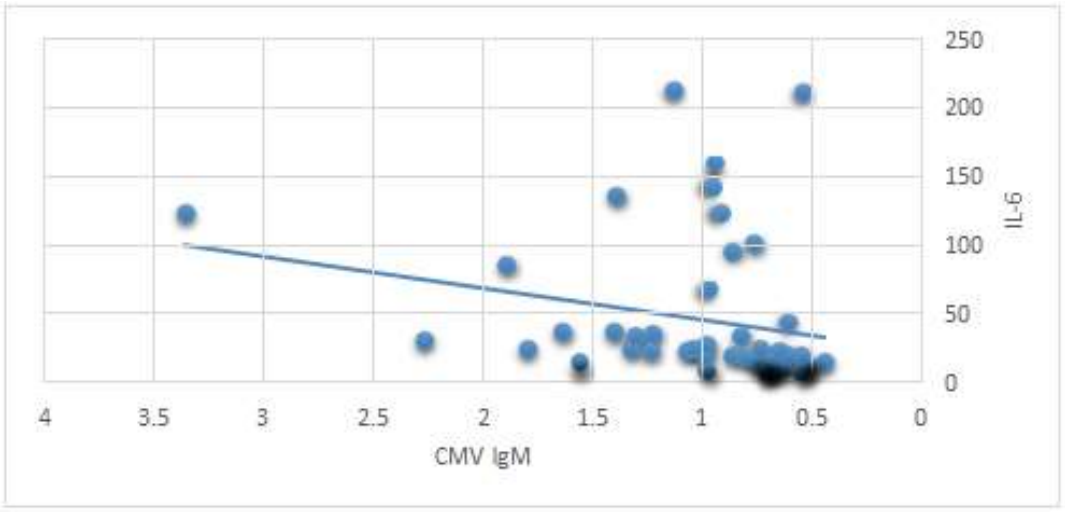

Figure-3. The cross plot between IL-6 and CMV IgM antibody.

Table 8 summarizes mean levels of Toxo and CMV within which significant differences were evident between Toxo IgM and Toxo-IgG at P<0.01. The same result regarding CMV IgM and IgG. Meanwhile, figure 4 represents a box plot using an ANOVA analysis of variance with repeated measures, showing a significant difference between the variables, F = 42.46, p = <.001 at p=0.05. Thus, the null hypothesis is rejected.

Table-8. The mean and standard error for patients' negatives for SARS2-Covid- 19 about *Toxoplasma gondii* and *Cytomegalovirus* IgM and IgG antibodies.

	CMV IgM	**CMV IgG**	**Toxoplasma IgG**	**Toxoplasma IgM**
Mean ± Std.	0.61 ± 0.1	0.96 ± 0.34	0.51 ± 0.09	0.83 ± 0.21

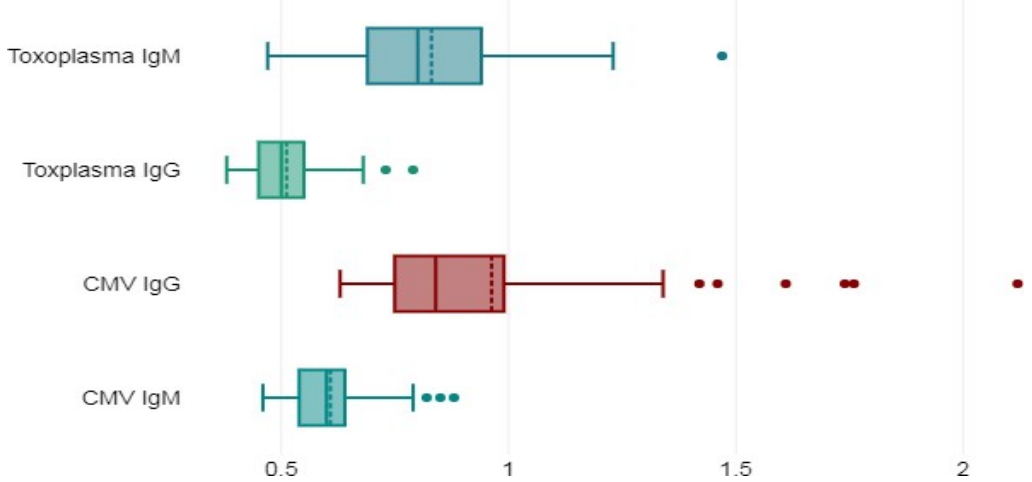

Figure 4. A box plot for Toxoplasma and CMV IgM and IgG antibodies in the control group.

DISCUSSION:

Since the 1960s, the province of Kirkuk has been plagued by several diseases considered endemic, including cholera, benign tertian malaria, cutaneous, visceral leishmaniasis, Toxoplasmosis, and other viral infections like rubella, CMV, and rota- virus. The migration of individuals from nearby provinces and even neighboring nations is a possible explanation for this phenomenon. The low-level infrastructure circumstances have arisen as a result of the events that occurred in 2003 and 2014, such as poverty and relocation. These reasons could play a part in the recent rise in epidemics, such as the high incidence or breakout of novel COVID-19 in Kirkuk Province

sometime around February 2020. The current study was shot to achieve the goal of a relationship between novel COVID and Toxoplasmosis, CMV infections.

According to Table 1, the CMV rate of 76.74 percent was high. This rate was significant because it was 20.34 percent IgM, indicating acute CMV infection. In contrast to a high IgG level, which implies two possibilities: protection against a past CMV infection or partial immunity, a low IgG level suggests neither protection nor immunity [18]. In this case, it is essential, especially in females, to monitor IgM elevation and to collect different IgG Abs folds. Most viral infections provide sterile lifelong immunity after infection [29].

Regarding females in the present study, it was hypothesized that pregnancy might reactivate the latent virus, leading to further reproductive losses. This result was less than that reported in Iraq by [29]. The difference may be due to the smaller sample size of 162 in the current study compared to 252 in the previous survey. Recent events after 2014 that destroyed the infra base and the migration of displaced people from neighboring provinces to Kirkuk Province have led to increased crowding and contamination by direct contact, especially for CMV. The present study's low immune status due to malnutrition may explain the high rate of CMV and co-infection with novel COVID-19.

The total toxoplasma rate in this research was 45.93 %, which is a high percentage. Due to the ongoing power outages that affected food storage and water supplies, as well as the lack of quality insecticides to kill the mechanical vector in Iraq and Kirkuk Province, this high rate may reflect environmental contamination with Toxoplasma oocysts [30]. The total rate of toxoplasmosis in the current research differs from that observed in the same province by [31,32,33], which recorded rates of 33.6%, 35.6%, and
31.15 %, respectively. Also, it differed from those reported [34,35,36], who documented corresponding rates of Toxoplasmosis in the same province of 92.1%, 48.9%, and 38.56%. This geographical frequency of *T. gondii* seropositivity was more significant than that seen in previous studies, including Egypt In Turkey, with 39.28 percent. Malaysia, India, Saudi Arabia, Mali, and northeast Thailand were cited in[18]. Also, it was much lower when compared to the 83.6 percent registered in Ethiopia [38]. Variations in the rates may be attributable to several variables, including sample size, laboratory procedure, research location, and patient type (infected or not infected with other infectious agents [37].

A negative correlation was observed between the presence of Toxo-IgG and CMV-IgG. It was also positive for IgM, with a mean value of 0.85 IU/ml compared to 0.48 IU/ml for Toxo- IgM. This study suggests that patients, particularly those childbearing age between 15 to 40, were more susceptible to CMV infection than toxoplasmosis. In addition, the CMV IgG level of 1.17 IU/ml was near the lower limit of the normal range of 1.0 IU/mL. Consequently, they must be partly immunized; notably, they are susceptible to infection when in contact with other pathogens, particularly new COVID-
19. Our findings agree with [38] and disagree with [39], who suggest a positive relation between Herpes simplex virus and SARS-CoV-2.

Concerning Toxo and CMV, males in the age range of 31 to 40 demonstrated significance when compared to males in other age groups; this result represents the degree of contamination and susceptibility to developing both protozoan and viral infection. In addition, employers in the Iraqi community spend a disproportionate amount of time outside for their age group, and they eat food that has been cooked outside, which may be a source of illnesses.

To explain the function of cytokines in the fight against *Toxoplasma gondii* infection. In a separate investigation into T cell production of IL-17 in resistance to *Toxoplasma gondii* in laboratory mice and the development of immune-mediated disease during this infection, it was reported that. The findings of this study cast doubt on the significance of *T. gondii*-identified N.K. cells as a major innate source of IL-17. The capacity of soluble

Toxoplasma Ag to stimulate N.K. cells to create IL-17 was reliant on the presence of accessory cells and the generation of IL-6, IL-23, and TGF-beta [40]. This was the case because soluble Toxoplasma Ag's ability depended on the presence of accessory cells. These findings point to the fact that many of the same cytokines that govern Th17 cells are also part of a pathway that regulates the innate production of IL-17. Furthermore, they point to a significant role of IL-6 in regulating the responses of N.K. cells. When it comes to CMV infection, it has been shown that the proinflammatory cytokine IL-6 is directly increased by CMV infection in endothelial cells. That CMV infection also upregulates the expression of the IL-6 gene. Additionally, IL-6 is the primary candidate found in the blood and connects local vessel-wall disease with systemic inflammation17 [41]. The following interpretations may be found in the previous sentence, along with the immune system weakening in older patients of both sexes. As a consequence of co-infections (Protozoa +Covid, CMV +Covid), and triple infections (Toxo + CMV +Covid), there was an increase in the amount of IL-6 found in the sera of SARS2-nCovid-19 participants in this investigation.

The results of IL-6 were in agreement with those reported in China by [40] and [41] in Egypt regarding the infection with new COVID-19. In contrast, the first report was IL-6 elevation among patients with toxoplasmosis and Cytomegalovirus during new COVID-19.

CONCLUSIONS

The rates of *T.gondii* and CMV among patients positive for SARS-2CoV 19 were high. Co-infection of SARS-2CoV-19 and CMV in females and the elderly had a substantial impact on the immune system, in particular, IL-6.

Further research is needed to clarify the role of IL-6 in severe inflammatory conditions, including COVID-19-related toxoplasmosis and CMV conditions, and to explore novel therapeutic options.

AUTHOR CONTRIBUTIONS

Amin and Salman designed the study. Jirjees and Ali perform the laboratory sampling and a few lab test procedures. Amin and Salman are responsible for data analysis and paper production. Amin and Salman proposed methods and interpretations for locating laboratories. Salman examined the document. Each author contributed to the article and approved the version that was submitted.

CONFLICT OF INTEREST

There were no commercial or financial ties that may be considered a possible conflict of interest throughout the study.

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