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EPIDEMIOLOGICAL ANALYSIS OF TOXOPLASMA GONDII AND COVID-19 SEROPREVALENCE IN CASES OF PREGNANCY LOSS IN KIRKUK CITY

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ABSTRACT

Background: Toxoplasma gondii is an require intracellular paramecium parasite accompanying a general distribution, contaminating an expansive range of warm-blooded animals and persons. Approximately individual-third of the worldwide community is estimated expected contaminated with T. gondii. In December 2019, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) arose and speedily spread worldwide, precipitating Coronavirus Disease 2019 (COVID-19). Both contaminations have existed associated with invulnerable dysregulation and antagonistic pregnancy effects, lifting concerns regarding their linked belongings during pregnancy.

Objective: To examine the partnership between Toxoplasma gondii and SARS-CoV-2 co-contamination and antagonistic gestation outcomes, accompanying particular prominence on the risk of failure and coagulation-connected deformities.

Methods: This case-control study judged serological and biochemical markers, containing cell with hemoglobin sedimentation rate (ESR), creatinine, C-reactive protein (CRP), ferritin, usually from breast dehydrogenase (LDH), aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), antagonistic-Toxoplasma IgM, and anti-Toxoplasma IgG. The calculated limits were analyzed to decide their friendship with gestation consequences among daughters contaminated with SARS-CoV-2 and T. gondii.

Results: Pregnant daughters with evidence of T. gondii contamination and COVID-19 manifested significantly bigger levels of inflammatory and clotting-connected biomarkers compared accompanying control groups. Co-contamination was associated with an raised frequency of unfavorable gestation outcomes, specifically miscarriage. Statistical reasoning disclosed a significant friendship betwixt co-infection rank, elevated instigative stones, and pregnancy obstacles.

Conclusion: Co-contamination with Toxoplasma gondii and SARS-CoV-2 grant permission influence an increased risk of antagonistic gestation outcomes through embellished instigative and coagulation disturbances. These verdicts climax the importance of early protect, careful dispassionate listening, and appropriate management of meaningful wives with doubtful co-contaminations to reduce motherly and before birth complications.

Keywords: Toxoplasma Gondii, SARS-Cov-2, COVID-19, Gestation Outcome, Failure, Co-Infection.

1 INTRODUCTION

Toxoplasma gondii is an demand intracellular minute organisms flunky and individual of ultimate good zoonotic pathogens general. Owing to allure curious organic changeability and complex internal clock, the sycophant is capable of contaminating nearly all warm-blooded mammals, containing guys. Members of the offspring Felidae symbolize the valid hosts and scrap environmentally antagonistic oocysts into the atmosphere, with facilitating widespread broadcast through blended snack, water, and soil. Human contamination occurs generally through the swallow of cloth cysts in not cooked center, devouring of cuisine or water devalued accompanying oocysts, inborn broadcast, finish transplantation, and blood transfer [1]. The ability of *T. gondii* to authenticate lasting secret contamination and conceal host invulnerable answers has handed out to its all-encompassing comprehensive disposal and ongoing society well-being significance.

Recent epidemiological studies have exhibited that toxoplasmosis waste highly approved all the while all of the world. Systematic reviews and meta-interpretations moved in Indonesia told dependable seroprevalence of antagonistic-Toxoplasma antibodies with the established society, establishing the extensive allocation of the attendant and its driven epidemiological burden [2]. Similar verdicts were stated in Iran, place a all-encompassing meta-reasoning recorded a extreme reign of *T. gondii* contamination and emphasize allure resumed importance as a society strength concern [3]. Furthermore, a systematic review from Pakistan pictorial big revealing rates among miscellaneous society groups, stressing the maintained broadcast of toxoplasmosis in native rules [4].

Pregnant daughters represent individual of ultimate unprotected cultures damaged by *T. gondii* adulteration. Primary maternal adulteration during pregnancy acknowledge likelihood result in transplacental broadcast of the leech and harsh before birth results, containing willing decline, stillbirth, congenital toxoplasmosis, hydrocephalus, chorioretinitis, moving animate nerve organs decay, and unending enlightening disadvantages [5].Epidemiological studies executed among significant wives in Zambia labeled significant seroprevalence and several risk cause guide motherly toxoplasmosis [6]. Likewise, studies from Pakistan stated solid revealing with two together pregnant and non-important mothers, indicating the resumed risk of contamination concurrently with an activity the generative ending [7]. Additional evidence from Poland recognized preventable environmental and regard manner of properlingy risk determinants guide motherly contamination, emphasize the significance of alive preventive patterns and community health attacks [8].

The worldwide importance of maternal toxoplasmosis has happened further habitual by studies from Brazil, which displayed a extreme prevalence of *T. gondii* contamination and frequent co-contamination accompanying TORCH pathogens among meaningful girls [9]. Similarly, investigations attended in Benin recorded ongoing broadcast and recently seized infections before birth [10], while research from Romania rooted persistent seroprevalence with girls of accouchement age [11]. Collectively, these findings stress the ongoing threat formal by toxoplasmosis to motherly and fetal well-being and support the exercise of improved screening and following programs.

In late 2019, the rise of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) surpassed to the Coronavirus Disease 2019 (COVID-19) pandemic, developing in exceptional global depression and humanness. Although originally recognized as a respiring affliction, COVID-19 is now implicit expected a complex multisystem disorder characterized by deep instigative answers, endothelial dysfunction, immune dysregulation, hypercoagulability, and multiorgan connection. Pregnant girls infected accompanying SARS-CoV-2 have existed proved to be at raised risk of failure, placental abnormalities, before birth development restriction, preterm beginning, and unfavorable neonatal consequences, making maternal COVID-19 a important dispassionate concern.

Growing evidence implies that incessant parasitic contaminations can influence susceptibility to fervid contaminations and alter ailment progress through timbre of host immune answers. Because *T. gondii* organizes persistent contamination and induces enduring changes in both inherited and adjusting exemption, considerable consideration has happened directed toward allure potential interplay with SARS-CoV-2. A current study from Northwestern Iran illustrated that the seroprevalence of T. gondii waited substantial two together before and all the while the COVID-19 pandemic, emphasize the ongoing epidemiological importance of toxoplasmosis regardless of the rise of SARS-CoV-2 [12]. Furthermore, a orderly review and meta-analysis stated a notable repetitiveness of *T. gondii* exposure between COVID-19 sufferers, suggesting a potential association middle from two points dormant toxoplasmosis and SARS-CoV-2 contamination [13].

Additional evidence advocating this relationship was determined apiece first case-control study transported in Iran, that demonstrated meaningful seroprevalence and genetic difference of *T. gondii* between COVID-19 patients [14]. Moreover, microscopic and immunological hearings among aborted wives with COVID-19 told simultaneous *T. gondii* infection, suggesting that co-contamination may help unfavorable pregnancy consequences through complex immunological and unhealthy mechanisms [15]. Recent Iraqi evidence more demonstrated the appearance of

antagonistic-*Toxoplasma gondii IgM* antibodies among inmates taking biological healing, emphasizing the resumed distribution of the parasite and allure dispassionate relevance in immunologically accessible populations [16].

Biologically, the interaction between *T. gondii* and SARS-CoV-2 seems plausible as both pathogens can cause massive immune activation, cytokine dysregulation, endothelial injury, oxidative stress, and coagulation abnormalities. Such parallel pathogenetic mechanisms may have a synergistic effect during pregnancy, leading to a stronger inflammatory response, thrombotic problems, altered placental function and miscarriage and other adverse pregnancy outcomes. Despite the increasing evidence of the individual effects of toxoplasmosis and COVID-19 in pregnancy, the impact of COVID-19 in the context of co-infection is poorly understood.

The current study aimed to explore the relationship between the two infections *(Toxoplasma gondii* and SARS-CoV-2) in pregnant women, and to evaluate the potential synergistic effect of co-infection on serological and immunological markers, coagulation related anomalies and negative pregnancy outcome, namely miscarriage. Understanding these relationships could improve risk assessment, aid early diagnosis, provide targeted surveillance, and help develop evidence-based care strategies for pregnant women at risk for these clinically important diseases.

2 MATERIALS AND METHODS

2.1 Equipment and Materials

The investigation was conducted with high performance laboratory diagnostic platforms, analytical instruments and equipment that met international standards of scientific correctness, reliability and reproducibility. The choice of equipment employed in this examination was carefully done on the basis of accuracy, reliability, analytical sensitivity and the frequent use of the equipment in biological studies and clinical diagnostics. To ensure a minimum margin of error in the measurements and to increase the validity of the data obtained, all high performance equipment was used. The following table provides detailed information of instruments, producers and origins. These proven technologies were integrated into the study, increasing the scientific quality, consistency and robustness of the results obtained.

Table 1: Equipment Specifications

S.No	Equipment Name	Manufacturer / Country
1	BS-240 Auto Analyzer	Mindray / India
2	Toxoplasma Cassette	Hitop / France
3	High-Speed Centrifuge	ALS LAB / China
4	Electrolyte Analyzer	Jokoh / India

All laboratory equipment used throughout this investigation has been consistently tested, maintained and checked for performance according to the manufacturers' specifications, until the end of the study. These quality assurance methods were put in place to check the accuracy and consistency of the data produced by the agents, so as to ensure optimal agent performance. Biochemical experiments were greatly enhanced by the BS-240 Auto Chemistry Analyser, which provided high throughput processing, outstanding accuracy and reliable analytical performance.

These advanced capabilities greatly facilitated the generation of accurate and repeatable biochemical documentation crucial for the effective execution of the investigation.BIOZEK is a global leading provider of laboratory supplies and the materials for diagnostics that were used in this inquiry were provided by BIOZEK. Careful piloting has been carried out to ensure that these issues are of good quality, consistent, reliable and suitable for the research's analytical methods. The use of standardised and verified procedures significantly improved the accuracy and repeatability of the laboratory results. The table below contains detailed information on materials, producers, and country of origin.

Table 2: Material Specifications

S.No	Material Name	Manufacturer / Country
1	Gel Tube	BIOZEK / China
2	EDTA Tube	BIOZEK / China
3	Sterile Syringe	BIOZEK / China
4	Tourniquet	BIOZEK / China
5	Nitrile Gloves	BIOZEK / China
6	Sterile Cotton	BIOZEK / China
7	Adhesive Plaster	BIOZEK / China
8	70% Alcohol	BIOZEK / China
9	Micropipette	BIOZEK / China
10	Tube Rack	BIOZEK / China
11	Yellow Tips	BIOZEK / China
12	Safety Disposal Box	BIOZEK / China

3 SAMPLE COLLECTION AND ANALYSIS

In the present study, the seroprevalence of *T. gondii* and the possible association with COVID-19 was investigated with 23 participants. Participants were then divided into clear groups based on the nature of their antibodies to *T. gondii* that were detected in the serological studies. The serum samples have been classified as having either beneficial antagonistic-*Toxoplasma* antibody for IgG antibody or an antagonistic antibody for IgM antibody or both IgG and IgM antibody, and these classes have been kept secret. This stratification allowed for an immunological assessment of the participants, and provided a framework for analysing the possible friendship between *T. gondii* infection and COVID-19 and its clinical and laboratory outcomes.

3.1 Blood Collection Protocol

Blood samples were drawn in a controlled aseptic environment, minimising the risk of environmental contamination and assuring sample integrity. The blood was collected from all participants in 2 mL of unaltered blood via non-sterile single-use syringes and needles. The ancestral samples were immediately moved into sterile coagulate retaining serum separator tubes for clot correction and best dispersion of anti-toxin. All the sample collection and management procedures were conducted by the laboratory with standard operating procedures and were applied to all participants to ensure reliability, sample quality and consistency of the reported samples.

3.2 Serum Separation and Preparation

The biological samples were then taken to the ALS Laboratory where they were processed and analyzed. The serum separation was carried out under specific operational conditions with a high-speed centrifuge and the spinning speed and duration were closely controlled to ensure proper separation of the serum from the constituents which formed naturally in it in accordance with known laboratory practice. Samples of antitoxin were tested for properties and accuracy before serological testing. All samples were promptly collected and treated after collection to preserve the integrity of the sample and the possibility of contamination was minimised, ensuring the accuracy of subsequent laboratory analysis. These protocols ensured that good quality samples of antitoxin could be obtained for dependable serological testing and accurate evaluation of the analysed biomarkers.

3.3 Toxoplasma Antibody Detection

In serological diagnosis, which is the most common method for the discovery of illness, an aliquot of the antiserum was carefully measured with a calibrated micropipette and dispensed onto the specified sample well of the Toxoplasma hasty test cassette (Hitop) per manufacturer instructions. All procedures were performed in patterned workshop settings for uniformity and authenticity of thickness in all verifiable samples. The Toxoplasma cassette was used to detect two ionized IgG and IgM against *Toxoplasma gondii*, the latter being highly specific for the detection of antagonistic antibodies, and from the extreme nervousness, it was used to identify them. Results obtained from this assay provided essential serological data to assess the predominance of contaminated by *T. gondii*, and to assess its potential friendship with the study variables.

3.4 Result Interpretation and Data Validation

The results of the tests were based on the visual assessment of the test and control lines on the assay cassette after the manufacturer's interpretation protocols. The specific test lines were used for detection of anti-*Toxoplasma gondii* IgG antibodies, anti-*Toxoplasma gondii* IgM antibodies, or both antibody classes were detected. All the samples were tested and in duplicate if necessary and the quality control was well monitored during the test to ensure accuracy, reliability and repeatability of the results.To avoid analytical errors and minimize potential observer bias, differences and ambiguous results were retested and results were independently assessed and verified by qualified laboratory staff.

3.5 Statistical Analysis

A statistical analysis was conducted to assess the relationship between the presence of Toxoplasma gondii antibodies, COVID-19 infection and adverse clinical outcomes such as miscarriage. Information regarding demographic, clinical and laboratory data were summarized by descriptive statistics and presented as frequencies, percentages, mean and standard deviation as appropriate. To discern differences and association between study variables, inferential statistical tests that include the chi-square test, independent samples test (t-test) and one-way analysis of variance (ANOVA) were used.

Potential confounding factors were adjusted for using multivariate logistic regression analysis in order to identify independent predictors of adverse clinical outcomes. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to express the strength of the associations. Kaplan–Meier survival curves and Cox proportional hazard regression models were used to perform survival analysis and estimate the time and hazard of miscarriage in the study groups for evaluation of time-to-event outcomes.

The statistical data was analyzed by using the standard analytical procedures using the [Statistical Software Name Version 26]. The p-value was discussed as two-tailed value less than 0.05 was considered statistically significant. The chosen statistical method helped to analyze the study objectives thoroughly, thereby improving the reliability and validity of the results.

4 RESULTS

Table 3 presents the disposal of *Toxoplasma gondii* seropositivity (IgG and/or IgM antibodies) in accordance with COVID-19 infection rank and group of same status among the study parties. The verdicts demonstrate the incident of *T. gondii* seropositivity in things the one were either COVID-19 positive or negative, providing awareness into the potential project between these contaminations across various age categories. The age range of the shareholders comprehensive from 18 to 58 age, indicating that two together young and middle-old adults can experience by *T. gondii* contamination and COVID-19.

Table 3: Toxoplasma and COVID-19 Detection Results

Patient Number	Toxo (IGm)	Toxo (IGg)	COVID-19 Status	Age (years)
A1	-ve	+ve	Positive	46
A2	+ve	-ve	Positive	33
A3	-ve	+ve	Positive	58
A4	+ve	-ve	Positive	31
A5	+ve	-ve	Positive	18
A6	+ve	+ve	Positive	27
A7	-ve	+ve	Positive	42
A8	+ve	-ve	Positive	21
A9	+ve	-ve	Positive	23
A10	-ve	+ve	Positive	44
A11	-ve	+ve	Positive	30
A12	-ve	+ve	Positive	34
A13	-ve	+ve	Positive	40

A14	+ve	+ve	Positive	26

The detection of antagonistic-*Toxoplasma* IgG antibodies plans previous uncovering or never-ending latent contamination, inasmuch as the ghost of IgM antibodies is indicative of a current or alive infection. The noticed allocation of IgG- and IgM-positive cases with earlier colleagues may indicate accruing exposure to the deadbeat over occasion as well as the incident of continuous contaminations within this group of same status. Moreover, there might be primary clinical associations between seropositivity for *T. gondii* and COVID-19, and the combination of the two may impact on the immune responses of the hosts and potentially on worse clinical energy outcomes.These rulings highlight the importance of accounting for possible contamination from *T. gondii* throughout the clinical thinking of objects related to COVID-19, especially those in high-risk groups such as women. Properly using two together, proper listening and proper management of two together contaminations can help to reduce the likelihood of negative clinical and gestation-accompanying outcomes.

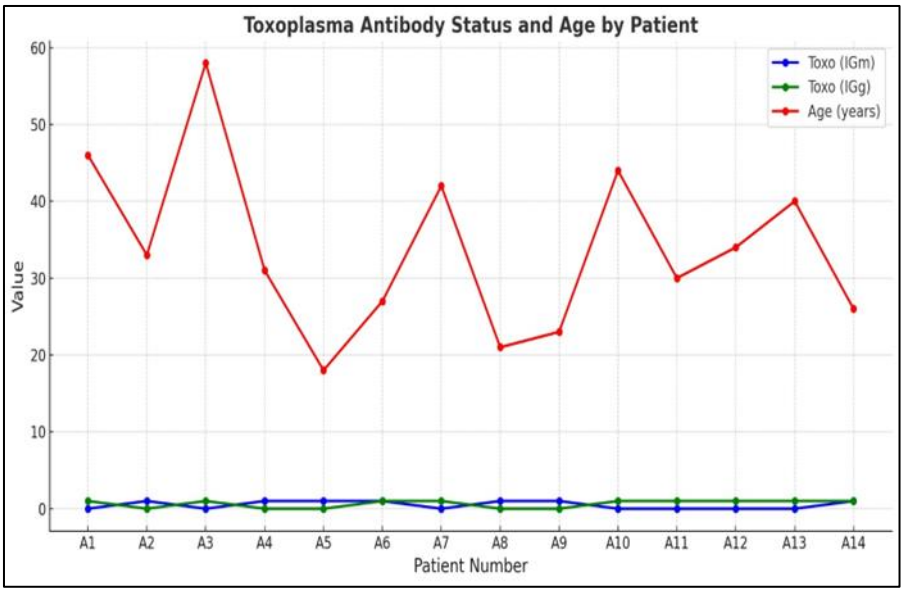


Figure 1: Toxoplasma Antibody Status and Age by Patient

Table 4 shows the distribution of angering and biochemical flags among the study groups. Increased levels of C-sensitive protein (CRP), ferritin, and typically of breast dehydrogenase (LDH) were seen, between those who had *T. gondii* and COVID-19 contaminations at the same time. The elevated levels of CRP suggest better basic inflammatory reaction, with the presence of contamination from alive invulnerable stimulation guide. Likewise, high levels of ferritin indicate an acute inflammatory response and high possibility levels indicate underlying inflammatory disease and disturbances in iron metabolism.

In addition, LDH levels were elevated in co-polluted persons, suggesting natural damage, fibre damage or increased metabolic stress due to spreading process. These biomarkers are at their maximum peak when they are co-contaminated with more than one bacterium, as compared to those contaminated by one bacterium.

These verdicts indicate that the middle to middle from two points *T. gondii* and SARS-CoV-2 may intensify inflammatory pathways, possibly in order to increase the danger of clinical snags. If the swelling and tissue damage that occurs by chance is excessive, a woman's level of significance for the disease is affected. Listening to the alarming biomarkers can thus give valuable information.

Liver function parameters were also altered in the study participants including those with simultaneous contaminations of *Toxoplasma gondii* and COVID-19 as demonstrated in Table 5. Elevated levels of aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) were noticed in several co-contaminated victims, indicating variable strengths of hepatic involvement. Possibility elevations tell of liver cell damage, liver stress or liver angering that fuels the infectious process.

The hepatocatalyst abnormalities seen during the observation may be attributed to the direct or indirect action of two or more pathogens on the liver tissue in combination with an integrated stimulatory response triggered throughout the infection. The location of *T. gondii* and SARS-CoV-2 grant permission further intensifying of angering and invulnerable-

arbitrated mechanisms, possibly resulting in enhanced hepatic dysfunction accompanying a single-bacterium contamination.

Table 4: Inflammatory Markers

Patient Number	CRP (mg/L)	Ferritin (ng/mL)	LDH (U/L)
A1	41.72	98.1	247
A2	24	218	293
A3	37.8	152	198
A4	31	88	160
A5	51	92	176
A6	49	197	262
A7	37	100	240
A8	30	122	183
A9	21	101	170
A10	33	255	292
A11	8.14	56.10	199
A12	60	173	209
A13	31.92	311	278
A14	59	187	192

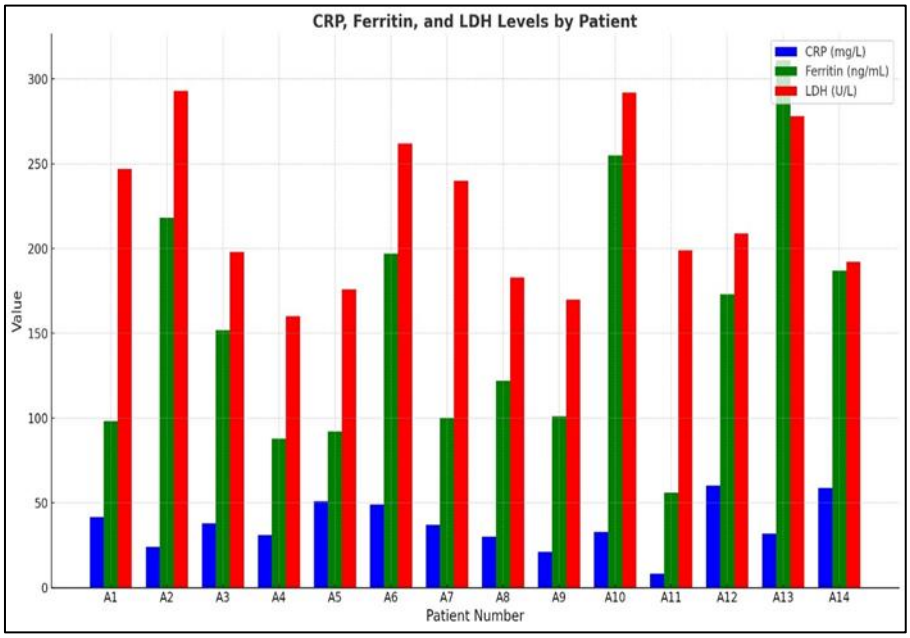


Figure 2: CRP, Ferritin&LDH Levels by Patient

These findings illustrate the importance of regular assessment of liver function tests in co-infected patients. The sooner the hepatic abnormalities are identified, the more they will be able to be handled in a detached manner, optimally, and without causing additional issues. Also, liver function impairment can affect the absorption of drugs and response to effect, highlighting the importance of careful, non-biased evaluation and effect in patients with liver damage.

Table 5: Liver Function Tests

Patient Number	GOT (U/L)	GPT (U/L)
A1	20	26
A2	16.22	27
A3	25.2	16.8
A4	20	31
A5	19	11
A6	47	31
A7	52	41
A8	14	11
A9	33	30.7
A10	51	40.5
A11	37	20.5
A12	47	33
A13	42	33
A14	21.7	18

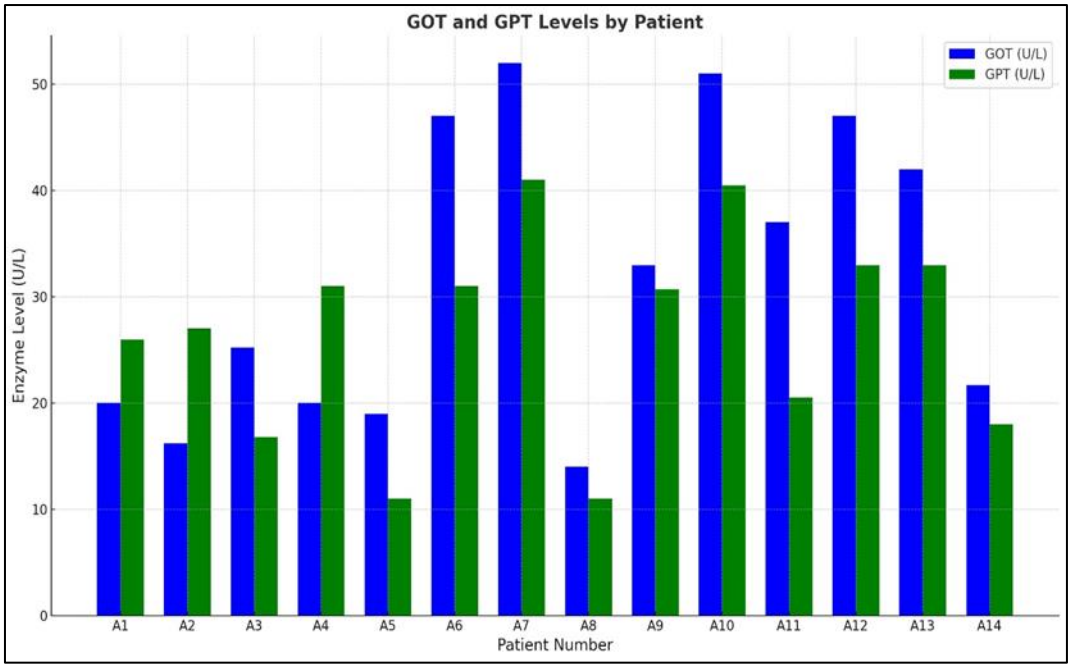


Figure 3: GOT and GPT Levels by patient

The renal function and cell with hemoglobin sedimentation rate (ESR) verdicts of the study parties are presented in Table 6. The results are related to different ESR and different antitoxin creatinine levels which were observed and explain the impact of infection on this level and the level of angering endeavor between the investigated groups.

The higher ESR principles found in the different parties indicate an ongoing angering reaction which corroborates evidence of higher immune activation found in different workshop parameters.The serum creatinine levels were variable among the subjects.

While few things-maintained creatinine concentrations inside the usual remark range, others shown exalted levels, suggesting attainable impairment of renal function. These judgments allow for the permission of variable renal engrossment related to the spreading process and then the instigative response.

The place of exalted ESR and increased creatinine levels in sure players grant permission reflect the fundamental character of the affliction and its potential belongings on diversified organ schemes. Persistent redness has happened associated with alterations in renal function and concede possibility influence sort injury resistant things.

Hence, routine monitoring of renal disease markers and clinical signs should be performed in order to detect renal impairment early and to guide informed and equitable treatment. An important point highlighted in the assessments in Table 6 is the importance of monitoring renal function and watch for warning signs in patients with infection with *Toxoplasma gondii*, COVID-19 or co-infection with both.

Table 6: Renal Function and ESR

Patient Number	ESR (mm/hr)	Creatinine (mg/dL)
A1	27	0.73
A2	60	0.90
A3	27	0.6
A4	21	0.5
A5	32	0.8
A6	39.51	0.92
A7	29	0.68
A8	29.11	0.54
A9	34.9	0.98
A10	38	1.09
A11	13	0.4
A12	33	0.91
A13	38	0.99
A14	25.11	0.7

Table 7 shows the detailed biochemical and hematological analysis of the measured parameters in the study groups, which helps explain the biochemical and hematological changes in the body that might occur due to co-infection of T. gondii, COVID-19, and their effect. There was no significant difference between the groups in terms of GOT (AST), GPT (ALT), ESR, and CRP levels (p-value: 0.550424, 0.171095, 0.119511 and 0.845528 respectively). The results of this study suggest there is insufficient variability in liver enzyme activity or some inflammatory markers to differentiate the study groups and may reflect normal biological variation or patient-specific factors more than disease-specific effects.

Several biomarkers, however, showed statistically significant changes, which may indicate clinically relevant changes in organ functions and systemic inflammatory status. The levels of serum creatinine were significantly different among the groups ($p = 0.020139$), suggesting that there may be some differences in renal involvement and kidney function. This is an interesting result that could be due to the extent of disease, inflammatory load, or physiological response in the subjects. In severe infectious diseases renal dysfunction has been noted as an important complication and a rise in creatinine in some groups may be the first indication of renal dysfunction and may warrant more intensive clinical management.

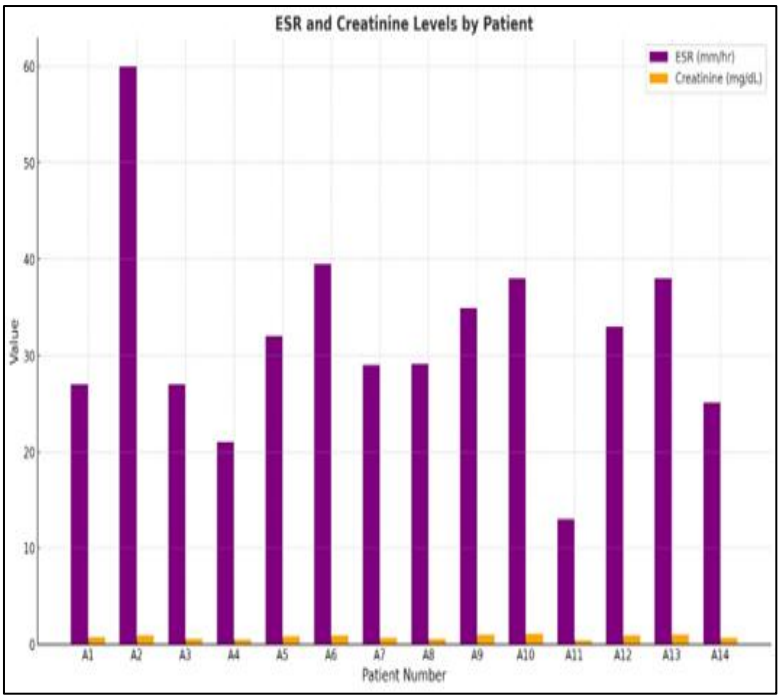


Figure 4: ESR & Creatinine Levels by Patient

Ferritin also showed a statistically significant difference (p = 0.009962), indicating significant differences in inflammatory activity and iron metabolism between the groups. Optimal ferritin levels are a well recognized acute-phase reactant, and increased levels are often related to overactive immune response and systemic inflammation. The observed difference indicates that there was an inflammatory response of a higher intensity in some participants, which could be involved in disease progression and affect clinical outcomes.

Among all the parameters assessed, there was the greatest difference between the groups when it came to lactate dehydrogenase (LDH) (p = 0.001086). High LDH levels are associated with tissue damage, increased cellular destruction and increased metabolic stress. The high differences in LDH activity indicate that there could have been some differences in extent of tissue injury and disease load in the subjects. This is important since LDH is a recognised indicator of the severity of viral and inflammatory illnesses.

Table 7: Statistical Analysis of Biochemical and Hematological Parameters

Variable	Mean	Median	Standard Deviation	Minimum	Maximum	P-value
GOT (U/L)	31.79	29.10	13.92	14.00	52.00	0.550424
GPT (U/L)	26.46	28.85	9.74	11.00	41.00	0.171095
ESR (mm/hr)	31.90	30.55	10.81	13.00	60.00	0.119511
Creatinine (mg/dL)	0.77	0.77	0.21	0.40	1.09	0.020139
CRP (mg/L)	36.76	35.00	14.57	8.14	60.00	0.845528
Ferritin (ng/mL)	153.59	137.00	73.33	56.10	311.00	0.009962
LDH (U/L)	221.36	204.00	46.39	160.00	293.00	0.001086

In addition to statistical significance, the range of the different laboratory variables offer valuable clues about the heterogeneity of the study population. Some biomarkers were fairly similar in all participants, while others exhibited significant variability, possibly due to variations in immune responses, inflammatory state, disease progression, and susceptibility of the individual. This diversity reinforces the complexity of the interactions between host and pathogen, and the importance of understanding laboratory results in the context of clinical history.

In general, the results of Table 7 revealed that no significant difference exists between liver enzymes and certain inflammatory markers of the study groups, while creatinine, ferritin and LDH levels were important markers of physiological disturbance and disease-related pathomorphological changes. These markers could be used as valuable

biomarkers of the renal involvement, systemic inflammatory response, and tissue damage in patients with infection caused by Toxoplasma gondii or COVID-19 or those infected with both. Compared with the significant changes in their external form, they have great potential for clinical application in disease monitoring, risk stratification, and the assessment of patient outcomes.

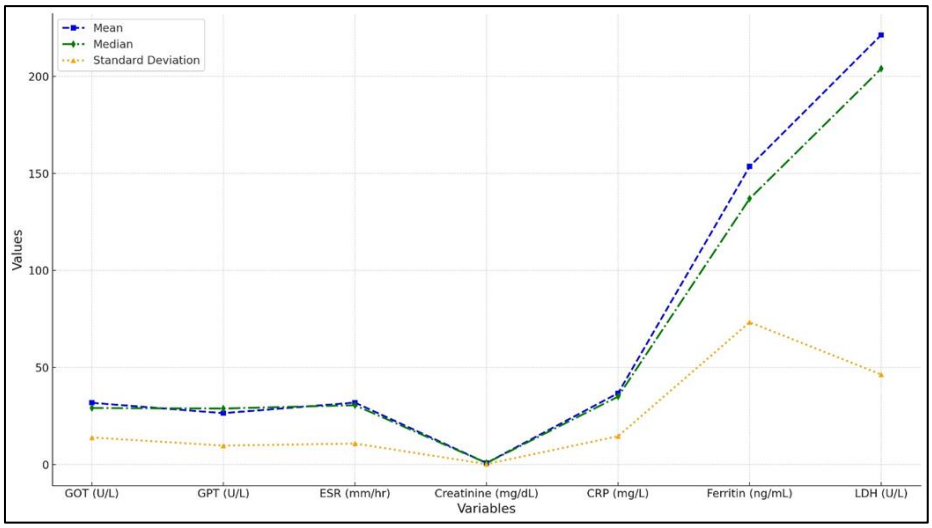


Figure 5: Statistical Analysis of Biochemical and Hematological Parameters

5 DISCUSSION

The results of the present study showed that *T. gondii* and SARS-CoV-2 coinfection could be correlated with the marked changes of inflammatory, hepatic, and renal biomarkers indicating possible interacting effect of both pathogens. These observations confirm the possibility that co-infection can lead to a more pronounced organismic reaction than individual infections with the two pathogens. The increase in the levels of CRP, ferritin and LDH in co-infected patients suggests that there is an increased inflammatory activity and tissue damage which suggests that there may be a synergy of inflammatory reactions between *T. gondii* and COVID-19 [18–22].

In severe COVID-19, patients have an overactive immune system leading to a release of high levels of pro-inflammatory cytokines and acute-phase reactants, which is often referred to as a cytokine storm [21]. High CRP levels have always been correlated with the severity of the disease and the poor clinical outcomes which indicate the severity of systemic inflammation [22]. Likewise, increased ferritin levels have been linked to hyperinflammatory responses and dysregulated iron metabolism in COVID-19 patients [23]. Increased LDH levels are also thought to represent cellular injury and tissue destruction and have been reported as independent markers of disease severity and mortality [24]. Elevated levels of these biomarkers in co-infected individuals in the present study indicated that *T. gondii* infection may exacerbate inflammatory and pathological responses when present in conjunction with other infections.

The observations could partly be due to the immunosuppressive effect of *T. gondii*. Toxoplasmosis has been shown in previous studies to induce a potent immune response of interferon-gamma (IFN-γ), tumour necrosis factor alpha (TNF-α) and other pro-inflammatory cytokines [25,26]. These cytokines are important in regulating the growth and spread of parasites and preventing disease, but ongoing immune activation can lead to chronic inflammation and immune dysfunction. Therefore, people who have a latent or active toxoplasmosis can have a heightened immune response after infection with SARS-CoV-2, which can result in more severe infection and a poorer clinical outcome [27]. More recently, the link between toxoplasmosis and COVID-19 has been investigated. There have been several reports of high seroprevalence of *T. gondii* infection among COVID-19 patients and possible impact of previous or concurrent toxoplasmosis infection on clinical course of SARS-CoV-2 infection [28, 29]. Furthermore, changes in cytokine levels of co-infected people have been linked with elevated inflammatory response, immune dysfunction, and disease burden [30]. The present study results corroborate this and support the hypothesis that a co-infection with *T. gondii* and SARS-CoV-2 might be another risk factor for poor clinical outcomes.

Another significant finding of this study was the change in liver function parameters in co-infected patients. High AST (GOT) and ALT (GPT) levels indicate liver involvement and indicate liver stress/injury. Liver dysfunction is well described as a symptom of COVID-19 infection, and occurs in a significant number of patients admitted to hospital [31]. Direct viral damage, general inflammation, hypoxic damage, endothelial dysfunction and hepatotoxic effects due to drugs have been suggested [32]. The current results indicate that simultaneous infection with *T. gondii* could further

exacerbate the effect of liver stress by other inflammatory and immune mechanisms. Thus, a liver enzyme abnormality seen in co-infected patients could be a result of the interaction of both infectious agents.

Another important finding of this study was the association with renal involvement. Some co-infected people had significantly elevated serum creatinine, suggesting that they may have kidney dysfunction. Severe COVID-19 patients have been shown to be at high risk for renal injury and have been found to have a higher mortality and morbidity rate [33]. Pathogenesis of renal dysfunction during SARS-CoV-2 infection is multifactorial, with direct renal viral injury, inflammatory injury, microvascular thrombosis and hemodynamic disturbances [34]. The present study revealed higher levels of creatinine, which indicates that the co-infection with *T. gondii* may be an additional stress on the kidneys, and increase the risk of developing acute kidney injury or long-term renal complications.

The data showed statistically significant differences between the research groups for all the evaluated biomarkers except for LFT.The variations between research groups were statistically significant for creatinine, ferritin and LDH but not for LFT. This suggests that they may be suitable markers of the severity and systemic involvement of the disease. Ferritin is an important measure of inflammatory burden and has been correlated with poorer outcome in severe infectious diseases [35]. Similarly, elevated LDH levels signify tissue damage and cellular injury, and have been shown to serve as a negative prognostic indicator in COVID-19 patients [36]. Increased levels of these biomarkers in co-infected persons reflect a possible higher degree of systemic stress and organ involvement.

The findings have important clinical implications, especially in pregnancy. Infection with toxoplasmosis during pregnancy can cause fetal abnormalities, miscarriage and poor outcomes for the newborn, and is a major cause of these problems in the world when the mother is infected congenitally [37].COVID-19 virus has been associated with pregnancy complications, thrombosis and placenta inflammation and dysfunction. A simultaneous infection may exacerbate the inflammatory and prothrombotic environment, hence increasing the risk of problems during pregnancy and fetal l distress. Therefore, accurate and sensitive screening and monitoring of all pregnant women who have been exposed to either infection might be necessary to reduce the risk to the mother and fetus.

The present study has several strengths, such as assessing inflammatory, hepatic and renal biomarkers in patients with *T. gondii* and COVID-19 at the same time. Some constraints need to be recognised, however. The sample size was relatively small which might decrease the generalizability of the study results, and serological approaches were not sufficient to differentiate between recent, chronic and reactivated toxoplasmosis. Future large-scale prospective studies with molecular diagnostics, cytokine profiles and prospective follow-up are suggested to further elucidate the biological interaction between *T. gondii* and SARS-CoV-2.

6 CONCLUSION

The results of the present study indicate that there may be a significant interaction between Toxoplasma gondii infection and COVID-19 with substantial changes in inflammatory, hepatic and renal biomarkers. Co-infected patients had significantly elevated laboratory markers of systemic inflammation, tissue damage and organ dysfunction such as C-reactive protein, ferritin, lactate dehydrogenase and serum creatinine levels. This aberrancy reflects the immune interaction between *T. gondii* and SARS-CoV-2, which could be associated with a heightened inflammatory reaction and additional physiological stress possibly leading to higher levels of clinical symptoms and health consequences.

The observed associations are consistent with the idea that latent or active toxoplasmosis could modulate immune responses to SARS-CoV-2 infection, and play an additional role in the course of the infection. A particular area of concern is the possible effect of co-infection on pregnant women, as there is a risk of increased inflammation and immune dysfunction that could lead to poor pregnancy outcomes such as miscarriage and other maternal-fetal issues.

The results have to be interpreted with caution because of the limitations of the study, but they reinforce the clinical significance of having to take into account the status of toxoplasmosis in patients who are infected with COVID-19 particularly those who are vulnerable. Laboratory blood markers of inflammation, liver and kidney function could be useful for risk stratification, disease monitoring and clinical decision making. Additional multicenter studies on a larger scale are needed to better understand the mechanisms of this interaction and its actual clinical and public health impact through the use of molecular diagnostics and advanced immunological investigations.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

Statement of ethical approval

This study was conducted in accordance with the ethical standards and received formal approval from the Institutional Ethics Committee of the University of Kirkuk.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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