The relation of some hematological parameters in Toxoplasma gondii infection associated with myocarditis

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Abstract

Background: Toxoplasmosis is an infective disease that originates from zoonotic transmission and is most dominant in countries with low socio-economic nations. Humans can acquire the disease via the consumption of infected food that contains the parasite oocyst or through the ingestion of the parasite tissue cyst in raw or undercooked meat. This study was designed to evaluate the seroprevalence of T. gondii in myocarditis patients and the alterations in some common hematological parameters and other inflammatory and cardiac biomarkers.

Materials and methods: The study was carried out in Kirkuk Governorate from March 2022 to June 2022, blood specimens were extracted from 45 myocarditis patients diagnosed on clinical bases at Azadi Teaching Hospital-cardiopulmonary resuscitation ward and 45 healthy control. Patients and control serum were assessed for Toxoplasma IgM, IgG quantitative and ELISA, Haematological investigation and infectious biomarkers like (CRP, myoglobin and Procalcitonin (PCT) tests were also conducted on the patients and control groups.

Results: The data recorded significant increment in cardiac markers including CRP, myoglobin and PCT test, with P= 0.0012, 0.001, and 0.001 respectively. In addition, a significant data was recorded in gender regarding IgG level P=0.0038.

Conclusions: The study concluded that toxoplasma screening in myocarditis patients could provide a clue of the presence of infection and the haematological as well as the other inflammatory markers may help in monitoring active toxoplasma infection.

Keywords: *Toxoplasma, CRP, PCT and Myoglobin.*

INTRODUCTION

Toxoplasmosis is an infectious disease originated from animals and causes tissue invasion. Toxoplasma gondii has a worldwide spread specifically in humid and hot areas and is capable to prosper in variable intermediate hosts with felidae being the final host for the parasite (1). The pathogen is usually transmissible and occur the most in

poor and low hygienic areas worldwide with elevated cases of infection in remote areas were animals are in close contact with people with low standard of sanitization (2). Generally, the infection modes of the parasite is achieved via digestion of raw uncooked or meat, contaminated water with the infective stage of the parasite acquired from feces of cat or tissue cyst in meat in addition to acquiring the organism vai blood transfusion form infected blood products (3). The pathogen can cause infection in both human and vertebrates and I is an intracellular parasite that reside in parasite vacuole (4). The microorganism can occupies wide range of body tissues including the heart muscle inducing pericarditis, myocarditis and disruption of the heart function (5). The term myocarditis refers to the inflammation of the heart which could arise from various infectious and non-infectious conditions. Myocarditis could be the result of the proliferation of the tachyzoit in heart tissue or the consequence of the inflammatory response activity (6). The typical symptoms of myocarditis in relation to T. gondii infection is the compliance of arrhythmias, cardiac failure, effusion, and pericarditis (7). The disease may present with lymph node enlargement as well as mild illness but in immunocompromised patients the sympotms could be serious result in brain and ocular infection (2). The immune response to T. gondii can develop antibodies against the pathogen and the appearance of IgM and IgG Abs could referee to the parasite being inducing either acute or chronic infection in the past or recent time (8). The immune response to the pathogen can vary depending on the severity of the infection as well as the host immune status and specific Abs can be detected specially IgA, IgM and IgG. The process of parasite proliferation inside the cells can consume vast amount of lipids in order to generate the vacule tha t enclose the pathogen within the cell, thus some biochemical and metabolic activities in the infected individuals could be detected and there is prominent link of the parasite activity and the function of the liver which can reflected on the liver enzyme function (9).

Materials and methods

The study was performed in Kirkuk city/Iraq during March 2022 to July 2022, where the original approvals were obtained from the Kirkuk Health Directorate at the beginning of April. One hundred eighty (180) specimens were extracted from patients and healthy sets of both sexes and of different ages at Azadi Teaching Hospital-cardiopulmonary resuscitation ward, from patients entering the ward on a daily basis and then cases of coronary heart disease, enlarged heart, and clots affecting the heart muscle were excluded. Questionnaire form designed by the investigator in parallel with approval form was filled and signed by each individual participant, and then only cases of coronary heart disease, enlarged heart, and clots affecting the heart muscle were excluded. Approximately 5 milliliters (ml) of whole blood sample was extracted from all patient, 2ml blood was left in EDTA tube for study of blood hemoglobin (Hb) concentration, total while cell count and platelet counts were performed, while 3ml blood was left in gel tube and left at room temperature then was spun at 2000 rpm for 10min. The serum was collected in Eppendorf tubes for later study to determine the Toxoplasma IgM, IgG quantitative and qualitative ELISA test and other disease biomarkers like (CRP, PCT, and myoglobin tests).

Results

The current data showed that the mean age of the patients was 64.29 years of which 27 (60%) males and 18 (40%) females, while the average healthy control age was 28 years, of which 17 (37%) males and 28 (62%) females as shown in Table (1 & 2). Most of the patients 28 (62%) from city center resident, whereas 17 (37%) from rural areas, as for the healthy group, 30 (66%) were city residents and 15 (33%) from rural areas, Table (2).

Table (1): Age distribution in study groups

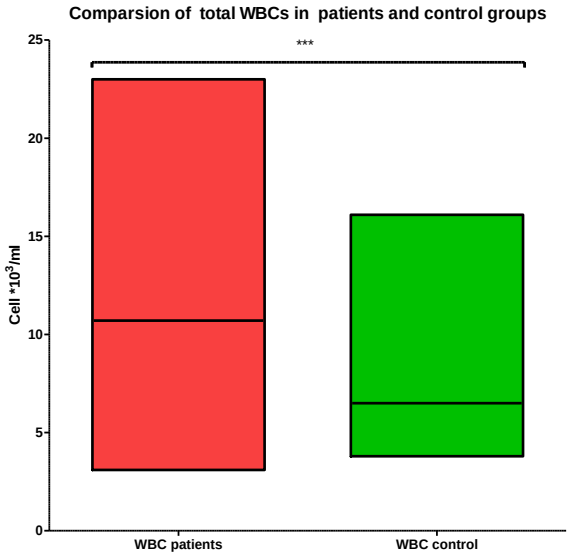
	Patients	Control
Age	Mean ±SD	Mean ±SD
	64.29±14.39	28.49±7.62
Total	45	45
	90	

Table (2): Gender and residency distribution in patients and control.

Profile		Patients		Control	
		No	**%**	**No**	**%**
Gender	Male	27	60	17	37.77
	Female	18	40	28	62.23
Total		45	100	45	100
Residency	Rural	17	37.77	15	33.33
	Urban	28	62.32	30	66.67
Total		45	100	45	100

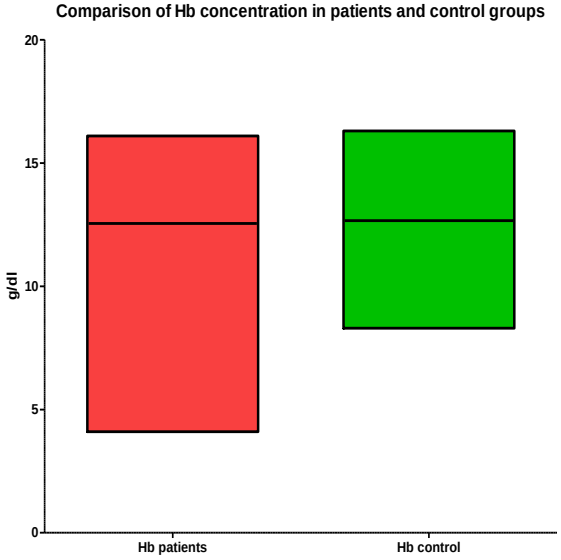
The current study showed that the mean and standard deviation (SD) of white blood cells in patients was 10.71, ±5.59, while in healthy subjects it was 8.24, ±3.63, accordingly, with significant difference (P=0.0005) between them when applying the Mann Whitney test as shown in Figure 1 & Table no. 3.

Figure (1) Displays difference between total WBCs in patients and healthy subjects.



The data of the illustrated here revealed that the mean and standard deviation (SD) for hemoglobin in patients were 12.56, ±2.18, while in healthy subjects it was 8.24, ±3.63, respectively, and no significant difference (P=0.8399) between the two groups when applying the Mann Whitney statistical test. as shown in Figure (2) and Table No. (3).

Figure (2) Displays difference between Hb concentration in patients and healthy subjects.



The data revealed here showed that the mean and standard deviation (SD) of blood platelets in patients was 266.5, ±111.8, while in healthy subjects it was 235.1, ±101.8, respectively, with no significant difference (P=0.2021) between them, at The Mann Whitney test is applied, as shown in Figure (3) and Table (3).

Figure (3) shows the difference between platelets count in patients and control.

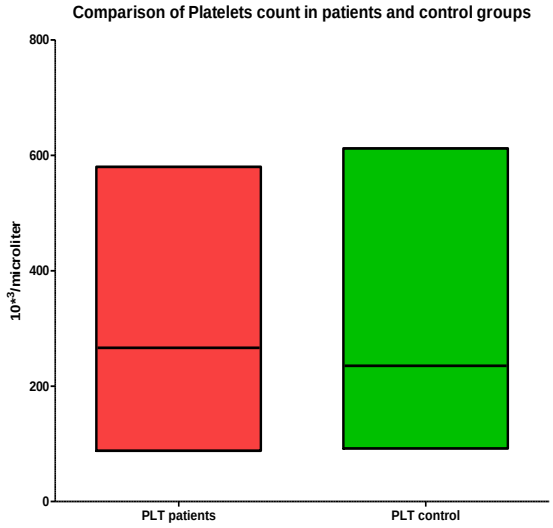


Table (3): Total count of white blood cell, hemoglobin and platelet.

Haematology parameters	Patients		Control		*P* value
	Mean	SD	Mean	SD	
WBC	10.71	5.59	8.24	3.63	0.0005
Hb	12.55	2.187	12.66	2.02	0.8399
PLT	266.5	111.8	235.1	101.8	0.2021
Total (n)	45		45		

The current study displayed that the mean and SD for C-reactive protein in patients were 13.0 ±12.9, while, in healthy subjects it was 5.6 ±2.3, accordingly, and significant difference (P = 0.0012) was obtained when applying Mann Whitney test as depicted in Table (4).

Also, the current data revealed that the mean and standard SD of myoglobulin in patients were 208.31± 78.32, while in healthy subjects 48.52±26.89, respectively, and significant difference (P=0.001) between the two groups was obtained when applying the Mann Whitney test, Table (4). On the other hand, the present study illustrated that the mean and SD for procalcitonin protein in patients were 3.98, ±1.9, while in healthy subjects 0.12, ±0.1, respectively, with significant difference (P=0.001) was recorded between the two groups when performing Mann Whitney test, Table (4).

Table (4): The CRP, myoglobulin and Procalcitonin levels in study groups.

Cardiac marker	Patients		Control		*P* value
	Mean	SD	Mean	SD	
CRP	13.0	12.9	5.6	2.3	0.0012
Total	45		45		
Myoglobulin	208.31	78.32	48.52	26.89	0.001
Total	45		45		
Procalcitonin	3.98	1.9	0.12	0.1	0.001
Total	45		45		

Regarding ELISA tests of Toxoplasma gondii, the number of myocarditis patients who had IgM Abs were 9 (20%) out of 45 patients, while these antibodies were not detected in any of the healthy subjects. While the number of patients who appeared to have IgG, antibodies were 12 (26.6%), whereas these antibodies appeared in only two patients (4.44%) of the healthy group, Table (5).

Table (5): Toxoplasma IgM and IgG Abs in patients and controls.

Toxoplasma ELISA	Patients				Control			
	Positive		Negative		Positive		Negative	
	No	%	No	%	No	%	No	%
IgM	9	20	36	80	0	0	45	100
IgG	12	26.6	33	73.4	2	4.44	43	95.56
Total	45				45			

The data of the present research revealed that the mean and SD of the IgM antibodies formed against Toxoplasma gondii in patients were 2.539 ±5.873, while these antibodies were not formed in healthy subjects, significant difference (P=0.0017) was obtained when applying Mann Whitney test Table (6). On the other hand, the mean and SD of IgG antibody against Toxoplasma gondii in patients were 3.31 ±6.74, while in healthy subjects it was 0.43 ±2.05, respectively, significant difference (P=0.0038) between

them was found after applying Mann Whitney test as displayed in Table (6).

Table (6) IgM and IgG antibodies for Toxoplasma gondii in patients and control groups.

Antibody type	Patients		Control		*P* value
	Mean	SD	Mean	SD	
IgM	2.539	5.873	0.0	0.0	0.0017
Total	45		45		
IgG	3.311	6.741	0.431	2.056	0.0038
Total	45		45		

Discussion

Toxoplasma gondii is a common parasite of human that has global dissemination. T. gondii infection in in immunocompetent subjects is mild but in immunodeficient people it can cause serious complications (10). With regard to the age factor, the average age of patients included in this research was 62 years, because most heart diseases begin to appear in advanced ages in life, and the causes of myocarditis vary according to the factors causing it. Preliminary results related to complete blood picture indicated that there were significant differences in the total white blood cell count in patients compare to healthy subjects, P = 0.005, which indicates a rise in the number of these cells in patients with myocarditis, which reflect the presence of a pathological injury that may result from active Toxoplasma gondii proliferation which can provoke the immune cells to propagate in order to control the parasite proliferation. However, the percentage of patients who had IgM antibodies were 20% of the total number, and approximately 27% of them had IgG antibodies, which indicates the presence of toxoplasmosis infection either in the acute or chronic form that may induce injury that could be one of the causes of myocarditis.

The current study found that the parameters of the blood picture related to the percentage of hemoglobin in the blood as well as the platelet count were not significantly differ from what is in healthy group, and this is due to the nature of toxoplasma disease which is not directly affected the tissues that generate blood cells, and the lack of significant differences indicates that this parameters does not influenced by toxoplasmosis in acute as well as in chronic infections (9). Raissi et al., recorded that the total white blood cell count rises with infection with toxoplasmosis, which is in line with the results of our study (11). In addition, Sandri et al., found that patients who had IgM antibodies directed against Toxoplasma gondii had an increase in total white cell count as a result of elevation in lymphocytes count (12). However, the decline in WBCs count could result from the influence of the parasites virulence techniques to modulate the effectiveness of humoral as well as cell mediated immune response to the parasite (9). In addition, the results of the current study are in agreement with the results of a study conducted in Basra, which included the observation of a high white blood cell count in patients with toxoplasmosis compared with healthy subjects who had no acute or chronic infection. The reason behind the high number of white cells in patients with toxoplasma gondii may be due to the general inflammatory condition, which includes the immune system's attempt to contain the spread

of the parasite (9) as this process may require the mobilization and proliferation of certain cell clones that act against the parasite's different stages.

The data of current study contradicted with of another study conducted in the Kingdom of Saudi Arabia by Ismail, which found that the total white blood cell count decreases after infection with toxoplasmosis (3). On the other hand, the average of white blood cells did not exceed the normal count of these cells, which indicates that the parasite's attempt to reduce its immune aggressiveness, which enables the parasite in its various stages to multiply and spread to other tissues, which explains the possibility of the parasite in its various stages to spread in areas far from the site of the initial infection (13). T. gondii has wide distribution in mammals and other hosts in addition to human and due to the nature of the infection not serious damage can generate in those hosts which allow them to be a harbor to disseminate the parasite and continue the life cycle (14).

Regarding CRP, the preliminary data of this research showed that the level of this protein was significantly higher (P= 0.0012) in patients group compared with the healthy control, these data are in parallel to similar research carried out on myocarditis associated with toxoplasma infection were significant elevation of CRP was reported in patient group (5). Yet, Sandri et al. stated that CRP is not significantly elevated in the case of toxoplasma infection (12). Concerning myoglobin and PCT, the current data revealed a significant increment of myoglobin activity and PCT in patient group compared to control (P=0.001). Previous report demonstrated a positive link of PCT levels and bacterial infection, in addition, this marker has not been elevated in vial induced diseases and this marker will drop to normal range after antibiotics been applied to cure the causative agent (15). Brancaccio et al., reported that higher muscle damage rate was recorded from T. gondii infection in immunosuppressed person. The parasite cystic stage (bradyzoite) can remain in tissue for extended time with less damage to the surrounding tissues (16). Yet, tissue destruction may arise due to mechanical pressure of the cyst or metabolic activity of the bradyzoite proliferation. The level of enzymes produced in skeletal muscles can vary accordingly according to certain conditions like physiological activities or in pathological changes. The e(16). The influence of tachyziote and bradyziot convertion in tissue has a great impact on the activity of many markers and invite studies was performed to assess these effects as it is easy to proliferate and cost effective as well as it can simulate the circumstances in vivo (17). In addition, our data is in consistent with Tveit et al. who reported significant rise in myogenic enzyme in cardiac attack patients (18). On the other hand, the data displayed here revealed that 20% of our patients were IgM positive which agrees with Alvarado et al. who reported 23.6% of IgM positivity in myocarditis patients (2). Whereas, IgG Abs was found in 26.6% of all the patients included in the research and 4.44% of control group which disagree with Khademvatan et al. who recorded 63.73% of all patients were positive for toxoplasmosis and in control group 37.64% were positive for IgG ELISA (19). Also, Testing for IgG Abs for toxoplasmosis cannot exclude pervious infection from that of reactivation and thus confirmation is necessary to sought out this issue. Regarding the cardiac biomarkers, these are nonspecific indicator of heart disease and not directly related to toxoplasma infection

further tests may be required in future studies to confirm such findings.

Conclusion:

T. gondii is a prevalent pathogen of animals as well as humans at a high frequency. The biomarkers of hematology as well as the inflammatory markers seems to be associated with the disease pathology though they are indirectly affected by the infection and they can provide useful indicators for the progression of the infection.

Reference

1. N. A. Ahmed, N. I. Mohamed and B. M. Raza. Gene sequence and Gene expression of IL-1β in aborted women infected with Toxoplasmosis. Teikyo Medical Journal. 2021,: 03875547 (44), 06
2. Alvarado-Esquivel C, Salcedo-Jaquez M, Sanchez-Anguiano LF, Hernandez-Tinoco J, Rabago-Sanchez E, Beristain-Garcia I, et al. Association between Toxoplasma gondii exposure and heart disease: A case-control study. J Clin Med Res. 2016;8(5):402.
3. Mohamed K. Hematological Changes during Chronic Toxoplasma gondii Infection in Pregnant Women in Makkah, Saudi Arabia. 2020;
4. Shehzad A, Masud A, Fatima T, Khan FM, Rehman S, Effendi MH, et al. Seroprevalence of Toxoplasma gondii and associated alterations in hematology and serum biochemistry of one-humped camels (Camelus dromedarius) in Pakistan. Vet World. 2022;15(1):110.
5. Babekir A, Mostafa S, Obeng-Gyasi E. The Association of Toxoplasma gondii IgG and Cardiovascular Biomarkers. Int J Environ Res Public Health. 2021 May;18(9).
6. Mustafa K, Hillyard J, Nowak E, Slowikowski J, Okogbue I, Garner D. Toxoplasma myocarditis: An atypical case in an immunocompetent patient. IDCases. 2021;26:e01273.
7. López‐López JP, Posada‐Martínez EL, Saldarriaga C, Wyss F, Ponte‐Negretti CI, Alexander B, et al. Tuberculosis and the heart. J Am Heart Assoc. 2021;10(7):e019435.
8. Xin K-S, Liu H, Wang H-B, Yao Z-L. Seroprevalence of Toxoplasma gondii among primary school children in Shandong Province, China. Korean J Parasitol. 2015;53(4):489.
9. Awad A, Ali A, Mahdi D. The effect of Toxoplasmosis on Hematological and Biochemical Parameters in Pregnant Women in Thi-Qar Province. 2020 Nov;889.
10. Daryani A, Sarvi S, Aarabi M, Mizani A, Ahmadpour E, Shokri A, et al. Seroprevalence of Toxoplasma gondii in the Iranian general population: a systematic review and meta-analysis. Acta Trop. 2014;137:185–94.
11. Raissi V, Bayat F, Taghipour A, Raiesi O, Ibrahim A, Getso M, et al. Seroepidemiology and risk factors of toxoplasmosis among children age ranged from 1 to 14 years referred to medical diagnostic laboratories in Southeast Iran. Clin Epidemiol Glob Heal. 2020;8(2):595–9.
12. Sandri V, Gonçalves IL, Machado das Neves G, Romani Paraboni ML. Diagnostic significance of C-reactive protein and hematological parameters in acute toxoplasmosis. J Parasit Dis Off organ Indian Soc Parasitol. 2020 Dec;44(4):785–93.

13. Harker KS, Ueno N, Lodoen MB. Toxoplasma gondii dissemination: a parasite's journey through the infected host. Parasite Immunol. 2015 Mar;37(3):141–9.

14. Matta SK, Rinkenberger N, Dunay IR, Sibley LD. Toxoplasma gondii infection and its implications within the central nervous system. Nat Rev Microbiol. 2021;19(7):467–80.

15. Cleland DA, Eranki AP. Procalcitonin. In: StatPearls [Internet]. StatPearls Publishing; 2021.

16. Brancaccio P, Lippi G, Maffulli N. Biochemical markers of muscular damage. Clin Chem Lab Med. 2010 Jun;48(6):757–67.

17. Guimarães EV, Carvalho L de, Barbosa HS. Interaction and cystogenesis of Toxoplasma gondii within skeletal muscle cells in vitro. Mem Inst Oswaldo Cruz. 2009;104:170–4.

18. Tveit SH, Myhre PL, Hanssen TA, Forsdahl SH, Iqbal A, Omland T, et al. Cardiac troponin I and T for ruling out coronary artery disease in suspected chronic coronary syndrome. Sci Rep. 2022;12(1):945.

19. Khademvatan S, Khademvatani K, Tappeh KH, Asadi N, Khezri P, Abasi E. Association of Toxoplasma gondii infection with cardiovascular diseases: a cross-sectional study among patients with heart failure diseases in Urmia, North-West of Iran. Ann Parasitol. 2020;66(2):193–9.