

The correlation of *Toxoplasma gondii* infection and the incidence of 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

Materials and methods: The present study was conducted in Kirkuk Governorate from April 2022 to August 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 qualitative ELISA test, and other cardiac biomarkers like (CRP, CK-MB, and Troponin tests).

Results: The data recorded significant increment in cardiac markers including CRP, CK-MB, and troponin with $P=0.0012$, <0.0001 , and <0.0001 respectively. In addition, a significant difference was recorded between males and females regarding IgG level $P=0.0038$.

Conclusions: The study concluded that screening for the presence of toxoplasma infection in myocarditis patients could provide a clear cut for the treatment regimen.

Keywords: *Toxoplasma, CK-MB, CRP, Troponin.*

INTRODUCTION

Toxoplasmosis is an infective disease that originate from zoonotic transmission and is mostly dominant in countries with low socio-economic nations. The disease is an obligated intracellular pathogen (1). Roughly the disease is estimated to affect one third of the globe population especially residence of subtropical and tropical regions and the infection rates at

highest levels in poor and low sanitation areas with dense population (2). Human can acquire the disease via the consumption of infected food that contain the parasite oocyst or through the ingestion of the parasite tissue cyst in raw or undercooked meat products like liver or steaks (3). The symptoms of the disease can widely varies between individuals which can be asymptomatic to sever illness in immune incompetent people (2). Following

consumption of the parasite containing infective stage, sporozoites release in the intestinal lumen and infect the lining epithelial of the gut releasing tachyzoites, the active form of the parasite, which cause more tissue invasion and damage. The tachyzoites are capable of inducing infection almost all nucleated cells and are capable to transmitted via infected macrophage to distant tissues and inducing infection and inflammatory process (4). The parasite sexual life cycle and final maturation occurs in the final host which are mainly the cats (5). Several influential factor affect the severity of the infection of most, the dosage of ingested parasite, the host immune condition, the genetic predisposition of the host as well as life style of the individual (6). The infection with this parasite can cause severe illness in immunocompromised patients or in the case of congenital infection with serious complications in fetal condition which could end up with loss of sight, retardation or even death (7). The infection in healthy people can be with mild symptoms like fever and lymph node enlargement, while in immune competent nervous system and heart tissue infection could arise (2). The infection with this parasite can lead to various tissue invasion like, muscles, liver, heart and brain and the parasite tend to encyst in tissues and remain dormant to cause later reactivation and tissue invasion (4). In addition, the pathogen tend to disseminate to cardiac muscle and induce pericarditis, cardiomyopathies and myocarditis (4). If the protozoan invade the heart muscle then arrhythmias may arise as well as pericarditis and cardiac effusion (2). Myocarditis due to toxoplasmosis could represent 12% of all cases especially in immunocompromised patients (8). A simple mean of detection of toxoplasmosis is indicated via the screening for antibodies (IgM and IgG) as the former antibody appear in early infection and represent

an acute infection, while the latter spike in the case of sub-acute or chronic infection (9). Nonetheless, the diagnosis of the infection must be confirmed with the presence of IgM antibody in the case of IgG Abs were found as the latter Abs may represent an old infection in the condition of treatment protocol was achieved (4,9). Other mean of indication of the infection can be established through some laboratory tests like ELISA, haemagglutination, indirect fluorescence antibody test (IFA) and latex test (10). In this article myocarditis patients will be assessed for the coexistence of toxoplasmosis acute infection using ELISA test to differentiate and assess the prevalence of the infection in those patients.

Materials and methods

The present study was carried out in Kirkuk Governorate from April 2022 to August 2022, after signed consent form were obtained from all participants, blood specimens were extracted from 45 myocarditis patients diagnosed on clinical bases at Azadi Teaching Hospital-cardiopulmonary resuscitation ward and 45 healthy control. Inclusion criteria included only myocarditis cases and other heart diseases were excluded like cases of coronary heart disease, enlarged heart and angina. Approximately 5 ml of venous blood sample was collected from each patient under sterile condition and left at room temperature to clot then centrifuged at 3000 rpm for 15 minutes. The serum stored collected in Eppendorf tubes and kept at -20 °C to determine Toxoplasma IgM, IgG quantitative and qualitative ELISA test and other cardiac biomarkers like (CRP, CK-MB, Troponin 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

Age	Patients	Control
	Mean \pm SD	Mean \pm SD
	64.29 \pm 14.39	28.49 \pm 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 SD for C-reactive protein in patients were 13.0 \pm 12.9, while, in healthy subjects it was 5.6 \pm 2.3, respectively, and there was a significant difference ($P = 0.0012$) between the two groups when applying Mann Whitney test as depicted in Figure (1) and Table (3).

Figure (1) Depicts the difference between C-reactive protein in patients and healthy subjects ($P = 0.0012$).

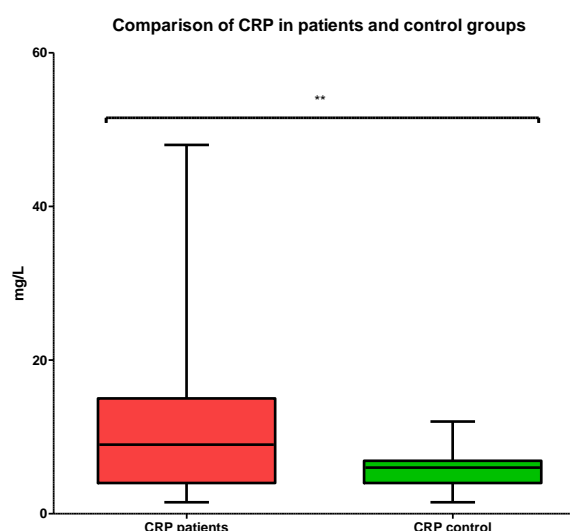
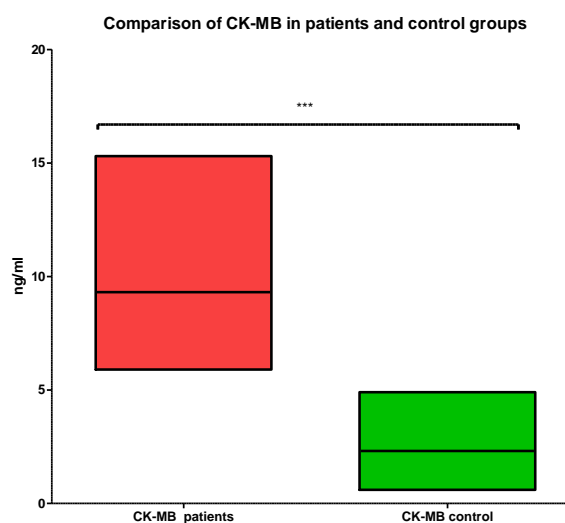


Table (3): The CRP levels in patients and control groups.

Cardiac marker	Patients		Control		P value
	Mean	SD	Mean	SD	
CRP	13.0	12.9	5.6	2.3	0.0012
Total	45		45		

The current data revealed that the mean and standard SD of creatinine kinase enzyme (CK-MB) in patients were 9.31 ± 2.60 , while in healthy subjects 2.31 ± 1.05 , respectively, and significant difference ($P < 0.0001$) between the two groups was obtained when applying the Mann Whitney test, Figure (2) and Table (4).

Figure (2) displays difference between CK-MB in patients and healthy subjects ($P = <0.0001$).

**Table (4): CK-MB differences in patients and control groups.**

Cardiac marker	Patients		Control		P value
	Mean	SD	Mean	SD	
CK-MB	9.31	2.60	2.31	1.05	<0.0001
Total	45		45		

The current study illustrated that the mean and SD for troponin protein in patients were $0.58, \pm 0.36$, while in healthy subjects $0.12, \pm 0.05$, respectively, significant difference ($P < 0.0001$) was recorded between the two groups when performing Mann Whitney test, Figure (3) and Table (5).

Figure (3) illustrates Troponin difference between patients and healthy subjects ($P = <0.0001$).

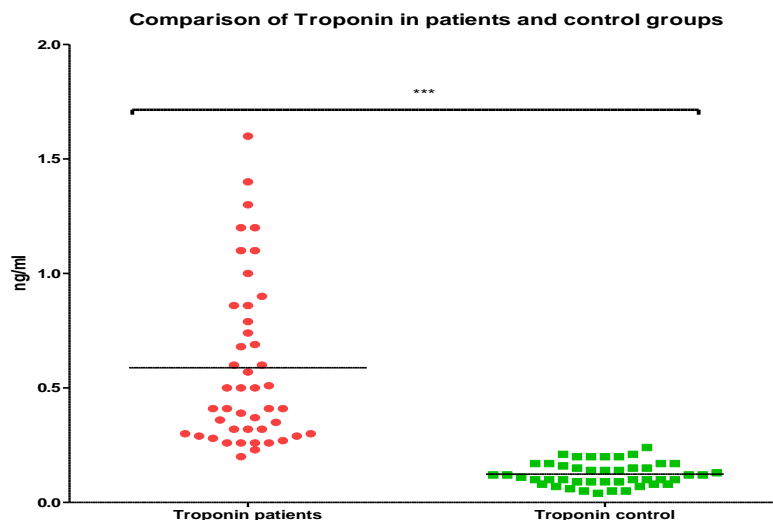


Table (5): Troponin differences between patients and control groups.

Cardiac marker	Patients		Control		P value
	Mean	SD	Mean	SD	
Troponin	0.59	0.4	0.12	0.1	<0.0001
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 (6).

Table (6): 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 results of the present research showed 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 ($P=0.0017$) between the two groups was obtained when applying Mann Whitney

test Table (7). 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 the two groups was found after applying Mann Whitney test as displayed in Table (7).

Table (7) 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		

Our data revealed that the number of male patients who had IgM antibodies were 7 patients, while patients with negative anti-*Toxoplasma* IgM Abs were 20 patients. However, in female patients, these antibodies were present in 2 patients and was not detected in the rest 16 patients. When applying the Q square statistical analysis, no significant difference was found ($P = 0.2790$) as illustrated in Figure (4).

According to IgG anti-*Toxoplasma* antibody, our data displayed that the number of male patients who had IgG antibodies were 10 patients, while the remaining 17 patients were negative. In female patients, these antibodies were present in 2 only, though it was not detected in the remaining 16 patients. When Q square was applied, no significant difference was detected between the compared groups ($P = 0.0540$), Figure (5).

Figure (4) the number of positive and negative IgM ELISA in relation to gender ($P = 0.2790$).

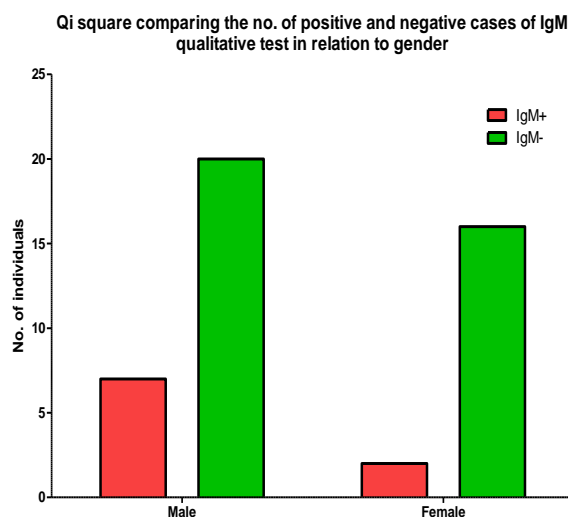
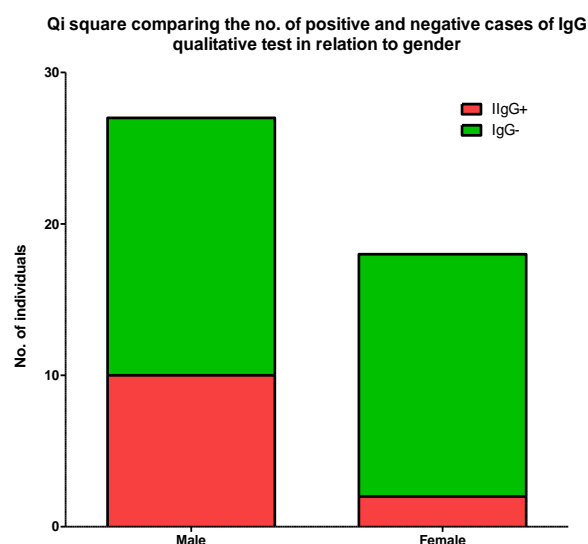


Figure (5) the number of positive and negative IgG ELISA in relation to gender (P = 0.0540).



Discussion

Establishing the differential diagnosis of myocarditis patient with screening cause for infection is quite useful especially in the case of *Toxoplasma gondii* antibody detection of IgM as it indicates the acute phase of the disease (11). Generally individuals with incompetent immunity tend to have persisted infection and chronic toxoplasmosis are more likely to develop latent infection (12).

As far as age is concerns, almost all the patients with myocarditis were average at 62 years indicating the development of cardiac issues at this age in our study population, nonetheless, the etiology for the myocarditis due to infectious cause must be assessed to rule out toxoplasmosis. Our data revealed that no local studies have linked toxoplasmosis infection with myocarditis.

Regarding to CRP, the preliminary results of the current study 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 similar research carried out on myocarditis associated with toxoplasma infection were significant elevation of CRP was reported in patient group (4). Also, our findings are in consistence with data reported from the USA revealed that elevated levels of CRP was associated with increase titer of IgG for toxoplasmosis (13). Yet, Sandri et al. stated that CRP is not significantly elevated in the case of toxoplasma infection (14). The reason behind the elevation in CRP in our data could be attributed to the fact that our patient group were admitted to intensive care unit on the bases of acute myocarditis and all patients were assessed for CRP and found to be rising at the time of admission. Those patient may have an acute inflammatory condition which may coexist with acute toxoplasmosis or even due to re activation of the infection in immunocompetent myocarditis, this elevation of this protein could indicated an acute condition which many be linked cytokine flare associated with toxoplasma infection (15). Also, infection with toxoplasmosis on the cellular level can surge oxidative stress on the surrounding tissues leading to enhancement of inflammatory cytokines like interferons and nuclear factor- κ B as well as other cytokines such as tumor necrosis factor and IL-12 which boost the secretion of CRP out of the liver cells (16).

On the other hand our data revealed a significant increment of creatinine kinase activity in patient group compared to control ($P<0.0001$). The elevation of this enzyme in the blood stream of patients could be explained by the fact that this enzyme is found in large amount inside the muscular cells as well as the brain and due to the parasite activity especially the invasion of heart muscle cells which lead to the parasite propagation intracellularly and eventually rupturing the infected cells and releasing vast amount of CK-MB enzyme in the

intercellular spaces which will then pass in to the circulation (17,18).

Concerning troponin, the results of the current research showed that the concentration of troponin rises much higher than in the healthy group, and significant difference between the two groups ($P < 0.001$) was recorded. These data are in parallel with Chen et al., who stated that troponin is elevated significantly in patients with myocarditis (19). In addition our data is in consistent with Tveit et al. who reported significant rise in troponin in cardiac attack patients (20). Likewise, troponin protein is principally abundant in heart muscles tissue and any damage to the cellular matrix of the heart cells induced by tachyzoites proliferation in these tissue will liberate troponin in vast amount in tissue spaces and eventually to blood stream (17). Depending on the extent of invasion involved the heart tissue, the more the tachyzoite activities inside heart muscle cells the extensive the infection and tissue damage is and ultimately more troponin to be released out of the cardiac cells (21).

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 (22).

Some of the drawbacks of this report are the relative small number of trail included in the research as large trail number will enhance the accreditiltiy as well as the statidstical power. Also, Testing for IgG Abs for toxoplasmosis can not exclude perviouss infection from that of

reactiavtion and thus confirmation is necessary to sough out this issue. Regarding the cardiac biomakers, these are non specificc indicator of heart disease and and not directly related to toxoplasma infection further tests may be required in future studies to confirme such findings.

Conclusions

The study concluded that toxoplasmosis could be one of the etiologies that give rise to acute myocarditis and screening for acute toxoplasmosis is crucial to establish accurate and effective treatment.

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