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Toxoplasma gondii infection among chronic hepatitis C patients: A case-control study

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ABSTRACT

Objective: To determine the detection rate of anti-*Toxoplasma gondii* (*T. gondii*) IgG and IgM in chronic HCV patients attending the Department of Tropical Medicine Mansoura University hospital in Egypt. **Methods:** This study included 120 adult chronic HCV patients, 81 decompensate cirrhosis (late-stage) and 39 chronic HCV non cirrhotic patients (early-stage) and 40 healthy blood donors as controls. Serum samples were examined for anti-*Toxoplasma* IgM and anti-*Toxoplasma* IgG antibodies by ELISA. Real-time RT-polymerase chain reaction assay was done for quantitation of hepatitis C virus. **Results:** Anti-*T. gondii* IgG antibodies were detected in 75 (92.6%) of 81 late-stage cirrhotic patients, 30 (76.9%) of the 39 chronic HCV non cirrhotic patients (early-stage) and in 6 (15%) of 40 controls with statistically significant difference (P<0.001). Anti-*T. gondii* IgM antibodies were found in 11 (13.6%) in late stage patients, 5 (12.8%) in early stage and in 3 (7.5%) of controls with no statistical significant difference (P=0.610). There was no correlation between stage of fibrosis and IgM or IgG antibodies positivity in our studied groups (P=0.526). High IgG levels significantly correlated with high viral load (P=0.026). **Conclusions:** Our findings suggest that the serious opportunistic *T. gondii* infection represent a potential significant risk for chronic HCV patients. So, toxoplasmosis should be considered in their investigations and follow-up.

1. Introduction

Toxoplasma gondii (*T. gondii*) is an opportunistic parasitic infection in immune compromised hosts and estimates indicate that up to one third of the world's human population is infected[1]. Although toxoplasmosis occurs worldwide, the seroprevalence of *T. gondii* infection can vary greatly between countries (10%-80%) and even within a given country[2,3]. People are infected by three principal routes of transmission: foodborne transmission (consuming undercooked, contaminated meat), animal-to-human transmission (ingesting oocysts shed in the feces of infected cats), and vertical transmission from mother to fetus through the placenta during pregnancy. Additionally, *T. gondii* can be transmitted via blood transfusion or organ transplantation from infected donors[4,5].

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Toxoplasmosis most commonly manifests as a mild, flu-like illness with low-grade fever, myalgia, malaise, and headache, but primary infection in humans may also cause spontaneous abortion, fetal mental and psychomotor retardation, retino-choroiditis, encephalitis, and hepatitis[6,7]. Infections with *T. gondii* are usually fatal in immunocompromised hosts unless the infection is recognized and treated[8]. Toxoplasmosis in the immunocompromised host is mostly due to the consequence of reactivation of disease acquired before immunosuppression occurred[9].

It is known that toxoplasmosis rarely leads to various liver pathologies, most common of which is granulomatous hepatitis in patients having normal immune systems. Patients who have cirrhosis of the liver are subjected to a variety of cellular as well as immunity disorders. Therefore, opportunistic toxoplasmosis can cause more frequent and severe diseases in patients with cirrhosis and is capable of changing the course of the disease[10].

Some studies have reported an increased prevalence of antibodies against *T. gondii* among liver disease

patients[10,11]. An association of *T. gondii* infection with liver cirrhosis has been reported in a previous epidemiological study[12].

Due to lack of information about the association of *Toxoplasma* infection and HCV infection in patients with chronic liver disease, this case-control study was performed to determine the seroprevalence of anti-*T. gondii* IgG and IgM levels in adult patients with chronic hepatitis C patients and to study the possible association of toxoplasmosis with deterioration of the liver condition.

2. Material and methods

2.1. Study population

A case control study was conducted from May 2010 through May 2012 at Tropical medicine Department, Mansoura University Hospital, Egypt. The study included 120 adult chronic liver disease patients, 81 HCV patients with decompensated cirrhosis (late-stage), 39 chronic HCV non cirrhotic patients (early-stage) and 40 healthy blood donors as controls from same region matched by gender and age. Clinical examination for the study subjects was done. Institutional ethical clearance and appropriate written informed consent was obtained from each participant.

2.2. ELISA for anti-Toxoplasma antibodies

Subjects of the study were examined with enzyme-linked immunoassays for anti-*Toxoplasma* IgG and anti-*Toxoplasma* IgM antibodies. Samples were tested for both anti-*T. gondii* IgM and IgG antibodies using (IMMUNOSPEC, CA, USA) kit which detects antibodies against highly purified native *Toxoplasma* antigens. In both procedures, the manufacturer guidelines were followed. For interpretation of semiquantitative detection of IgG-class antibodies to *T. gondii*, a graph is constructed by blotting the U/mL against the average OD of the controls; when the OD of the samples is reported on the graph, the U/mL contained in the serum samples can be calculated. A standard curve must be performed for each run. The results are expressed in IU and a result equal or greater than 10 U/mL was considered positive.

2.3. Real-Time PCR for early stage HCV patients

HCV RNA was extracted from 140 μL of each serum sample using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. RNA was eluted in 60 μL of elution buffer and stored at -20 ℃. Quantification of extracted HCV RNA were performed on a Mx3000P™ Real-Time PCR System (Stratagene, La Jolla, CA) using a *Taq*Man® OneStep RT-PCR Master Mix Reagents Kit (Applied Biosystems, Foster City, CA). HCV RNA was amplified according to the following program: 1 cycle each of 48 ℃ for 30 min and 95 ℃ for 10 min, followed by 40 cycles each of 95 ℃ for 15 s and 60 ℃ for 1 min. The threshold cycle values from samples were plotted on the standard curve and the numbers of copies were automatically calculated. For each run, positive and negative controls were included[13].

2.4. Liver biopsy, this was done for the early stage HCV patients

Liver biopsy was performed according to the consensus recommendations of the Asian Pacific Association for the Study of the Liver[14,15]. Written consent was taken from all patients before taking the liver biopsy. Liver histology was graded and staged according to Ishak fibrosis scaling system that classifies fibrosis into five stages (F0-F4) and activity into four grades A0-A3[16].

2.5. Statistical analysis

Data entry and analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL.). Summaries were presented in terms of mean, range and percentage as appropriate. Differences in proportions were evaluated by Pearson's *Chi*-square test. A statistical test result was considered significant whenever a $P<0.05$.

3. Results

A total of 120 adult chronic liver disease patients, 81 decompensate cirrhosis (late-stage) with mean age (52.33±6.76) years, 39 chronic HCV non cirrhotic patients (early-stage) with mean age (43.44±8.58) years in addition to 40 healthy blood donors as controls with mean age of (40.67±11.00) years were enrolled in this study. Clinical and laboratory data are shown in (Table 1&2). Clinically, the difference in the liver and spleen size between groups was statistically significant, $P<0.001$. No correlation was detected among groups regarding ascites or focal lesion. Concerning liver function tests (ALT, AST, serum albumin, billirubin level and prothrombine time); significant correlation was detected only between billirubin level and anti-*T. gondii* IgG in the late group ($r=0.245$ & $P=0.027$).

Anti-*T. gondii* IgG antibodies were detected in 75 (92.6%) of 81 late-stage cirrhotic patients, 30 (76.9%) of the 39 chronic HCV non cirrhotic patients (early-stage) and in 6 (15%) of 40 controls (Table 3). There is a statistically significance between positivity of anti-*T. gondii* IgG antibodies between 3 groups ($P<0.001$). In the late-stage group; of the 75 anti-*T. gondii* IgG positive patients, 32 (39.5%) had IgG levels higher than 150 IU/mL suggestive of prior infection, and 29 (35.8%) between 8 to 99 IU/mL. In the early-stage group; of the 30 anti-*T. gondii* IgG positive patients, 17 (43.6%) had IgG levels higher than 150 IU/mL, and 7 (17.9%) between 8 to 99 IU/mL.

Anti-*T. gondii* IgM antibodies were detected in 11 (13.6%) in late stage patients, in 5 early stage-patients (12.8%) and in

3 (7.5%) of controls with no statistical significant difference, P=0.610 (Table 3).

There was no correlation between stage of fibrosis and presence of IgM or IgG antibodies levels in our studied groups (P=0.576 and 0.526 respectively) (Tables 4). Presence of high IgG levels was significantly correlated with high viral loads levels (P=0.026) (Table 5).

4. Discussion

Toxoplasmosis is a protozoan disease that is widespread all over the world and demonstrates varying clinical manifestations. Determination of its incidence in various risk groups in the society and establishment of these risk groups play a significant role in taking the necessary precautions against this disease[12].

In this study *Toxoplasma* ELISA antibody positivity was significantly higher in cirrhotic patients. We found a higher frequency of anti-*T. gondii* IgG and IgM antibodies in chronic HCV patients than healthy blood donors controls. Previous reports about the association of *T. gondii* infection in liver disease patients in Egypt and other countries are scarce. The high level of anti-*T. gondii* IgM and IgG levels in cirrhotic patients than in healthy control may be explained by the occurrence of humoral and cell-mediated immunity defects in chronically infected patients with consequent reactivation of latent infection[17,18]. Our results

Table 1
Clinical and demographic data of chronic HCV patients and control.

Variable		Groups			Total
		Late (%)	Early (%)	Control (%)	
Focal lesion	Negative	65 (45.1)	39 (27.1)	40 (27.8)	144
	Positive	16 (100.0)	–	–	16
Splenomegaly*	Negative	9 (11.3)	31 (38.8)	40 (50.0)	80
	Mild	57 (90.5)	6 (9.5)	–	63
	Moderate	10 (83.3)	2 (16.7)	–	12
	Sever	5 (100.0)	–	–	5
Ascitis	Negative	27 (25.5)	39 (36.8)	40 (37.7)	106
	Mild	15 (100.0)	–	–	15
	Moderate	22 (100.0)	–	–	22
	Sever	17 (100.0)	–	–	17
Liver**	Average	32 (28.8)	39 (35.1)	40 (36.0)	111
	Enlarged	3 (100.0)	–	–	3
	Shrunken	46 (100.0)	–	–	46

*There a statistical significance between groups regarding splenomegaly (P<0.001), **There a statistical significance between groups regarding liver size (P<0.001).

Table 2
Laboratory data of chronic HCV patients and control.

Variable		Mean	Std. deviation	Std. error	95% confidence interval for means		P value
					Lower bound	Upper bound	
Albumin	Late	2.771	0.655	0.073	2.626	2.916	0.001
	Early	4.228	0.465	0.074	4.077	4.378	
Bilirubin	Late	2.565	1.661	0.184	2.198	2.932	0.001
	Early	0.805	0.194	0.031	0.742	0.868	
ALT	Late	41.803	25.574	2.842	36.148	47.457	0.260
	Early	47.974	32.400	5.188	37.472	58.477	
AST	Late	57.370	32.513	3.613	50.181	64.560	0.068
	Early	45.923	30.462	4.878	36.049	55.798	
PT	Late	64.593	13.802	1.534	61.541	67.645	0.001
	Early	86.487	10.190	1.632	83.184	89.790	

Table 3
Anti-*T. gondii* IgG and IgM antibodies in the study groups.

Group	IgG		IgM	
	Positive(%)	Negative (%)	Positive (%)	Negative (%)
Early (n=39)	30(76.9)	9(23.1)	5(12.8)	34(87.2)
Late (n=81)	75(92.6)	6(7.4)	11(13.6)	70(86.4)
Control (n=40)	6(15.0)	34(85.0)	3(7.5)	37(92.5)
P value	0.001		0.610	

Table 4
Correlation between stage of fibrosis and presence of IgM & IgG antibodies.

Liver fibrosis	IgM		IgG		Total
	Negative	Positive	Negative	Positive	
Mild (F1-F2) (%) *n*=27	23(85.2)	4(14.8)	7(25.9)	20(74.1)	27(100.0)
Severe (F3-F4)(%) *n*=12	11(91.7)	1(8.3)	2(16.7)	10(83.3)	12(100.0)
Total	34	5	9	30	39
P value	0.576		0.526		

Table 5
Correlation between viral loads levels and presence of IgG and IgM antibodies.

Viral load	IgG		IgM		Total
	Negative	Positive	Negative	Positive	
Low (< 100.000) *n*(%)	1(11.1)	5(16.7)	3(8.8)	3(60.0)	6(15.4)
Mild (100.000 -400.000) *n*(%)	5(55.6)	3(10.0)	7(20.6)	1(20.0)	8(20.5)
Moderate (400.000-800.000) *n*(%)	2(22.2)	9(30.0)	10(29.4)	1(20.0)	11(28.2)
High (>800.000) *n*(%)	1(11.1)	13(43.3)	14(41.2)	0(0.0)	14(35.9)
Total *n*(%)	9(100.0)	30(100.0)	34(100.0)	5(100.0)	39(100.0)
P value	0.026		0.022		

coincides with that reported in an early Egyptian study where a 65.5% seroprevalence of *T. gondii* antibodies were detected in patients with acute and chronic hepatic diseases against a 27% seroprevalence found in controls[19] and a Turkish study where researchers found an association of *T. gondii* infection with liver cirrhosis[12]. Our results conflict with those reported in a Mexican study where most of their patients suffered from liver disese but they did not find any association between seropositivity to *T. gondii* and cirrhosis with comparable seroprevalence of *T. gondii* IgM and IgG levels in patients and controls[10]. This difference could be attributed to the inclusion of liver disease patients due to many causes (alcoholic and HCV liver cirrhosis, steatosis, chronic hepatitis, acute hepatitis and amoebic liver abscess) and not confined to only HCV infected group in that study. Each cause of liver disease has different impact on immunological and pathological sequel.

In this study, 6 (15%) out of the 40 blood donor control, had anti-*T. gondii* IgG, 3 (7.5%) of them had IgG levels higher than 150 IU/mL, and 9 (22.5 %) between 8 to 99 IU/mL. Compared to other countries, our overall seroprevalence within blood donors was lower than seroprevalence reported in blood donors from countries including Mexico[20], Brazil[21], Chile[22], Malaysia[23], India[24], Egypt[25] and New Zealand[26], where seroprevalences varied from 20.3% to 75.0%. The relatively low overall seroprevalence in Egypt is probably because the majority of the population eats well-cooked food.

As blood transfusion is a potential transmission route for *Toxoplasma* infection, some studies suggest that toxoplasmosis transmitted through blood transfusion could lead to serious clinical consequences in immunocompromised patients and multiple blood transfusion recipients. Patients with liver cirrhosis are more susceptible to this risk in particular as they may need frequent blood transfusion for correction of severe anemia or hemodynamic resuscitation following repeated attacks of haematemesis[27]. However, routine screening for *T. gondii* in blood and blood products is not mandatory in Egypt. In our study, 3 (7.5%) of controls blood donors had IgM antibodies against *T. gondii*. This may one of the explanations for the high prevalence of anti *T. gondii* IgG and IgM in our cirrhotic patients. Therefore, blood donors with an acute phase of infection could be excluded from donation especially in absence of exclusion of *Toxoplasma* parasitemia by real-time PCR. This may be a good preventive measure especially if the recipient is an immunocompromised like cirrhotic patients[28].

The current study revealed that there was no correlation between stage of fibrosis and presence of IgM or IgG antibodies. Our results does not support the concept that the number of activated hepatic satellite cells (HSCs, which is responsible for hepatic fibrosis) was significantly higher in the *T. gondii* infection groups than that in the healthy group which may represent an active role of HSCs in liver pathology and the pathobiology of *T. gondii*-related hepatitis[29]. In the current study, compensated early cirrhotic patients showed high IgG levels that were significantly correlated with high viral loads levels (P=0.026), while moderate and high viral load levels were significantly correlated with absence of IgM antibodies (P=0.026).

These results are supported by the fact that depletion of CD4 and CD8 T cells during chronic infection with viruses like HCV results in parasite reactivation[30,31], and it may be the same etiology causing high viral replication in our chronic HCV patients with high IgG titer. While the association of low viral load and negative IgM levels are sign of both viral and parasitic clearance and this may be a reflection to the immune status of the patients. It is worth mentioning that patients with AIDS and serum anti-HCV positivity had an increased prevalence of antibodies against hepatitis B virus, *T. gondii* and Cytomegalovirus as opposed to a lower frequency of serum autoantibodies[11].

This study highlights that *T. gondii* is more prevalent among cirrhotic patients than in healthy blood donors. Since blood transfusion is a potential transmission route for

Toxoplasma infection, patients with liver cirrhosis are likely to form a *Toxoplasma* risk group.

Limitations of the study: repeated investigations following anti-*Toxoplasma*-treatment, especially viral load, needs to be done in patients with both high viral load and high *Toxoplasma* antibodies, to confirm this comorbidity.

Conflict of interest statement

We declare that we have no conflict of interest.

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