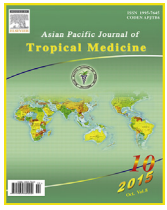
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2015.09.003

Is toxoplasmosis a potential risk factor for liver cirrhosis?

Abeer Abd El-Rehim El-Henawy[1], Ahmed Abdel-Razik[2], Sahar Zakaria[2], Dina Elhammady[2], Niveen Saudy[3], Manar Sobh Azab[1*]

[1]*Department of Medical Parasitology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt*

[2]*Department of Tropical Medicine, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt*

[3]*Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt*

ARTICLE INFO

Article history:
Received 15 Jul 2015
Received in revised form 20 Aug 2015
Accepted 15 Sep 2015
Available online 25 Sep 2015

Keywords:
Toxoplasma gondii
HBV
HCV
Liver cirrhosis
METAVIR score
Child-Pugh score

ABSTRACT

Objective: To document *Toxoplasma gondii* (*T. gondii*) antibody status in patients with liver disease, blood samples were taken from 180 hepatic patients and 180 healthy controls.

Methods: *Toxoplasma* IgG antibody was detected using enzyme-linked immunosorbent assay and histopathological assessment of liver biopsy METAVIR score was applied.

Results: Anti-*T. gondii* IgG antibodies were found in 32.8% of patients and in 22.2% of controls (P = 0.02). *Toxoplasma* seropositivity was significantly associated with lymphadenopathy, history of blood transfusion and reflex impairment in patients. Chronic hepatitis C virus (HCV) and chronic HCV-related cirrhosis groups compared to chronic HBV and chronic HBV-related cirrhosis groups expressed significantly higher prevalence of *T. gondii* seropositivity (odds ratio (OR) = 4; 95% confidence interval (CI): 1.3–12.6; P = 0.013, OR = 4.8; 95% CI: 1.5–14.9; P = 0.006, respectively). Within the chronic HCV group, *T. gondii* seropositivity significantly associated disease evolution as regards to METAVIR histopathological system for fibrosis and inflammation (OR = 19.4; 95% CI: 2.3–165.2; P = 0.0008, OR = 0.29; 95% CI: 0.1–0.8; P = 0.01, respectively). Albumin, international normalized ratio (INR) and platelets count were the laboratory parameters significantly altered in *Toxoplasma*-positive chronic HCV patients (P = 0.001, 0.03, 0.04, respectively). Child-Pugh scoring for cirrhosis in chronic HCV group placed the majority of seropositive patient in class C with significant statistical difference compared to Child A reference group (OR = 0.08; 95% CI: 0.01–0.5; P = 0.003).

Conclusions: *Toxoplasma* seropositivity was high in patients with cirrhosis and associated higher grades of inflammation and necrosis signifying disease evolution, suggesting that cirrhotic patients may thus form a risk group for toxoplasmosis.

1. Introduction

Toxoplasmosis is a parasitic zoonosis with the highest human incidence [1]. Seroprevalence of *Toxoplasma* infection among immunocompromised patients is high and reactivation of latent infections in them can be life-threatening [2]. During chronic toxoplasmosis, both $CD4^+$ and $CD8^+$ T lymphocytes are required to prevent reactivation of toxoplasmosis. Thus, depletion of T cells in the setting of chronic infection as in depressed cellular immunity states, leads to reactivation of latent infection [3]. Cirrhosis is considered an immunocompromised state that leads to a variety of infections, which then account for an approximate 30% mortality [4].

Hepatic involvement in toxoplasmosis does exist but it may go unnoticed as infection spreads to the liver early in course of infection and may not induce laboratory or clinical alterations [5]. Granulomatous hepatitis [5], hepatomegaly, abnormal liver function tests [6], cholestatic jaundice [7], cirrhosis [8] as well as liver dysfunction in liver and kidney transplant recipients [9] are the usually reported consequences. Hepatitis in *Toxoplasma* infection varies between 11% and 89% depending on the virulence of the strain [10].

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic viruses affecting about 600 million individuals

*Corresponding author: Manar Sobh Azab, Department of Medical Parasitology, Faculty of Medicine, Mansoura University, 2 El-Gomhouria Street, Mansoura 35516, Egypt.
Tel: +2 0122 3930 535
E-mails: manaryym@yahoo.com, msazab@mans.edu.eg

Peer review under responsibility of Hainan Medical College.

worldwide. Severe liver diseases, such as liver cirrhosis and hepatocellular carcinoma are usual consequences of chronic infections resulting in 1 million deaths per year [11]. *Toxoplasma* and hepatitis viruses are intracellular pathogens that both stimulate polarised immune responses involving Th1 cytokine profiles (pro-inflammatory mediators) such as IL-12, IFN-γ and nitric oxide [12]. In developing countries, co-infection by more than one pathogen is widespread and it remains an underestimated risk factor for infection although it can play a critical role in the infection outcome via effects on the host immune response or by inducing changes in host physiology [13].

Globally, the prevalence of latent *Toxoplasma* infection among patients with hepatitis B and C viruses as well as cirrhotic patients has not been extensively investigated, and the effect of co-infections with these pathogens on the progression of liver disease needs to be clarified especially in Egypt where all of these pathogens are reported with high prevalence rates. The paucity of information motivated us to undertake this study to determine the prevalence of latent *Toxoplasma* infection among chronic viral hepatitis as well as cirrhotic patients compared to controls not complaining of liver disease and to explore the effects of co-infection on the course of liver disease within these patient groups.

2. Materials and methods

2.1. Participants

A comparative cross-sectional study was performed at Mansoura University Hospital, Egypt, during the period May 2013 and January 2014. One hundred and eighty patients with chronic HCV or chronic HBV, who attended the Tropical Medicine Department [67 females and 113 males, with mean age ±SD of (50.14 ± 12.60) years], participated in the study. Exclusion criteria included patients with schistosomiasis, heart failure, diabetes mellitus, hypertension, hyperlipidemia, peripheral vascular disease, hematological and neoplastic disorders. None of patients had received anticoagulant medications, non-steroidal anti-inflammatory drugs or oral contraceptive drugs before hospital admission. Patient groups were matched regarding age, gender and residence with 180 healthy controls [70 females and 110 males with mean age ± SD of (48.0 ± 7.5) years].

Patients were divided into 4 groups: Group I;: chronic HCV (n = 75 patients); Group II: chronic HCV-related cirrhosis (n = 45 patients); Group III: chronic HBV (n = 36 patients); Group IV: chronic HBV-related cirrhosis (n = 24 patients).

2.2. Ethical aspects

This study was approved by the Ethical Committee of Mansoura University. All participants were acquainted with the study and gave informed consent to participate in it after fully explaining the aim of the study to them. The study was conducted in accordance with the guidelines of the Helsinki Declaration.

2.3. Laboratory tests

Five mL of venous blood were withdrawn from patients and control participants and were divided into 3 aliquots: the first aliquot was used for liver function tests [ALT, AST, ALP, serum albumin, bilirubin, international normalized ratio (INR)], CBC and indirect haemagglutination test (IHA) for schistosomiasis to detect prior schistosomal infection, while the second was used for serum HBV surface antigen (HBs Ag) and HCV detection by PCR, and the third was analyzed for anti-*Toxoplasma* IgG antibody detection, using commercial *Toxoplama* IgG detection kit (DS-EIA-Anti-Toxo-G-Fast, DSI, Italy).

For *Toxoplasma* antibody testing, blood samples were processed immediately by centrifugation at 4000 rpm for 5 min after which they were kept at −20 °C until analysis. Anti-*Toxoplasma gondii* (*T. gondii*) IgG antibody levels were expressed as international units (IU)/mL, and a result greater than 15.95 IU/mL was considered positive. All tests were performed following the instructions of the manufacturer.

2.4. Histopathology of percutaneous ultrasound guided liver biopsy

Liver biopsies were analysed after paraffin embedding. Five μm sections were obtained from chronic HCV group, for hematoxylin and eosin (H&E) and Masson's trichrome staining. Each liver tissue sample was diagnosed on the basis of the presence of at least 10 complete portal tracts, which has long been considered the ‘gold standard’ to determine liver histology, disease activity and liver fibrosis [14]. The degree of histologic hepatic fibrosis and inflammation was scored using the METAVIR scoring system [15]. Based on the degree of lymphocyte infiltration and hepatocyte necrosis, the level of inflammation was classified from A0 to A3, with a higher score indicating more severe inflammation. Fibrosis was graded from F0 to F4 as follows: F0: no fibrosis, F1: portal fibrosis without septa, F2: portal fibrosis with rare septa, F3: numerous septa without cirrhosis, and F4: cirrhosis. Steatosis was quantified as the percentage of hepatocytes that contained fat droplets and classified into three groups: <5%, 5%–30% and >30% [16]. Liver biopsy was assessed by a pathologist blinded to clinical and laboratory data. All demographic and laboratory data were collected at the time of the liver biopsy.

2.5. Statistical analysis

To test for normal distribution, frequency of data was plotted against normal distribution curve. All data were parametric as most of the quantitative data showed normal distribution using Kolmogrov–smirnov test to test for normality. Frequency, mean, standard deviation were used to describe data. *Chi*-square test was used to test for association between *Toxoplasma* infection and sociodemographic and clinical characteristics. A student's t-test was used to compare the means between groups. A P value < 0.05 was considered statistically significant. These tests were run on an IBM compatible personal computer using the Statistical Package for Social Scientists for windows Ver. 20 (SPSS Inc., Chicago, IL, USA).

3. Results

Anti-*T. gondii* IgG antibodies were found in 59 (32.8%) of 180 patients and in 40 (22.2%) of 180 controls (P = 0.02). Of the anti-*T. gondii* IgG positive patients, 23 (39%) had IgG levels

Table 1

Demographic and clinical data of *T. gondii* seropositive against seronegative patients.

Characteristic	Seropositives (n = 59) [n (%)]	Seronegatives (n = 121) [n (%)]	*OR* (95% *CI*)	χ^2	*P*
Gender					
Male (n = 113)	36 (61.0)	77 (63.6)	0.9 (0.7–1.7)	0.12	0.73
Female (n = 67)	23 (38.9)	44 (36.4)			
Age groups (years)					
≤30 (n = 20)	10 (16.9)	10 (8.3)	2.3 (0.8–6.4)	2.30	0.10
31-50 (n = 90)	28 (47.5)	62 (51.2)	1.1 (0.5–2.0)	0.02	0.88
≥50 (n = 70, r**)	21 (35.5)	49 (40.5)			
Residence area					
Urban (n = 120)	43 (72.9)	77 (63.6)	1.5 (0.8–3.0)	1.50	0.22
Rural (n = 60)	16 (27.1)	44 (36.4)			
Socio-economic level					
Low (n = 131)	41 (69.5)	90 (74.4)	0.8 (0.4–1.6)	0.48	0.49
Medium (n = 49)	18 (30.5)	31 (25.6)			
Educational level					
Illiterate (n = 12)	6 (10.2)	6 (4.9)	1.9 (0.45–7.80)	0.76	0.40
Pre-high (n = 145)	45 (76.2)	100 (82.6)	1.2 (0.47–3.00)	0.13	0.70
High (n = 23, r**)	8 (13.6)	15 (12.4)			
Occupation					
Jobless (n = 63)	17 (28.8)	46 (38.0)	0.7 (0.3–1.3)	1.50	0.24
Worker (n = 117)	42 (71.2)	75 (62.0.)			
Lymphadenopathy					
Yes (n = 46)	22 (37.3)	24 (19.8)	2.4 (1.2–4.8)	6.40	0.01*
No (n = 134)	37 (62.7)	97 (80.2)			
Blood transfusion					
Yes (n = 67)	33 (55.9)	34 (28.1)	3.2 (1.7–6.2)	13.10	0.0003*
No (n = 113)	26 (44.1)	87 (71.9)			
Reflex impairment[a,b]					
Yes (n = 36)	21 (35.6)	15 (12.4)	3.9 (1.8–8.3)	13.30	0.0003*
No (n = 144)	38 (64.4)	106 (87.6)			
Visual impairment[b]					
Yes (n = 45)	18 (30.5)	27 (22.3)	1.5 (0.76–3.00)	1.40	0.23
No (n = 135)	41 (69.5)	94 (77.7)			

*Statistical significance at $P < 0.05$, **reference group.

[a] Biceps, triceps, knee jerk, ankle jerk and Babinski's sign.

[b] Testing was done according to the regulation of ministry of health prior to interferon therapy.

higher than 100 IU/mL, and 36 (61%) showed levels between 16 and 99 IU/mL. In comparison 18 (45%) of the anti-*T. gondii* IgG positive controls showed IgG levels higher than 100 IU/mL, and 22 (55%) expressed levels between 16 and 99 IU/mL. Anti-*T. gondii* IgG levels were comparable (P = 0.50) among patients and controls (data not shown).

Table 2

Seroprevalence of *T. gondii* infection in patient groups.

Patient category	Seropositives [n (%)]	Seronegatives [n (%)]	*OR* (95% *CI*)	χ^2	*P*
Chronic HCV (n = 75)	25 (33.3)	50 (66.7)	4.0 (1.3–12.6)	6.2	0.013*
Chronic HBV (n = 36)	4 (11.1)	32 (88.9)			
Chronic HCV-related cirrhosis (n = 45)	25 (55.6)	20 (44.4)	4.8 (1.5–14.9)	7.7	0.006*
Chronic HBV-related cirrhosis (n = 24)	5 (20.8)	19 (79.2)			

*Statistical significance at $P < 0.05$.

As Table 1 implies, none of the sociodemographic characteristics showed statistically significant association to *Toxoplasma* seropositivity in patient groups. Among clinical data, lymphadenopathy, history of blood transfusion and reflex impairment were significantly higher in seropositive patients [odds ratio (*OR*) = 2.4; 95% confidence interval (*CI*): 1.2–4.8; P = 0.01, *OR* = 3.2; 95% *CI*: 1.7–6.2; P = 0.0003, *OR* = 3.9; 95% *CI*: 1.8–8.3; P = 0.0003, respectively].

Table 2 shows the statistically significant difference between seroprevalence of anti-*T. gondii* IgG in chronic HCV and chronic HCV-related cirrhosis groups compared to chronic HBV and chronic HBV-related cirrhosis groups (*OR* = 4.0; 95% *CI*: 1.3–12.6; P = 0.013, *OR* = 4.8; 95% *CI*: 1.5–14.9; P = 0.006, respectively). Interestingly, within the chronic HCV group, a significant difference in seroprevalence of *T. gondii* as regards to METAVIR scoring was observed (Table 3). *T. gondii* infection was positive in 68% (17/25) of patients with late fibrosis (F3 + F4) with significant statistical difference compared to F0 reference group (*OR* = 19.40; 95% *CI*: 2.3–165.2; P = 0.0008), while positivity was 28% in early fibrosis cases (F1 + F2). Similarly, most of *T. gondii* positive cases (68%) were METAVIR activity (A2-A3) compared to 32% for METAVIR activity (A0-A1) with statistical significance (*OR* = 0.29; 95% *CI*: 0.1–0.8; P = 0.01). In accordance, within the same METAVIR scoring for fibrosis, higher degree and extent of necrosis marked liver biopsies of *Toxoplasma* infected (Figures 1B and 2B) compared to *Toxoplasma* uninfected chronic HCV patients (Figures 1A and 2A).

Table 3

Liver biopsy METAVIR scores in chronic HCV patients according to *Toxoplasma* serostatus.

Liver biopsy	Seropositives (n = 25) [n (%)]	Seronegatives (n = 50) [n (%)]	*OR* (95% *CI*)	χ^2	*P*
Pathology scores					
F0 (r**)	1 (4%)	16 (32%)			
Early fibrosis (F1 + F2)	7 (28%)	20 (40%)	5.0 (0.6–50.3)	2.8	0.09
Late fibrosis (F3 + F4)	17 (68%)	14 (28%)	19.4 (2.3–165.2)	11.2	0.0008*
Activity scores					
A0- A1	8 (32%)	31 (62%)	0.29 (0.1–0.8)	6.0	0.01*
A2- A3	17 (68%)	19 (38%)			

*Statistical significance at $P < 0.05$, **reference group.

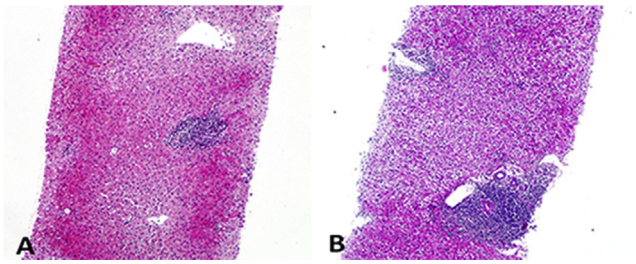


Figure 1. Liver biopsy with METAVIR fibrosis score 1 of (A) chronic HCV-*Toxoplasma* seronegative patient showing portal lymphocytic infiltrate with mild piece meal necrosis and no lobular necrosis (activity score 1); (B) chronic HCV-*Toxoplasma* seropositive patient showing portal lymphocytic infiltrate with moderate piece meal necrosis and mild lobular necrosis (activity score 2).
H & E ×100.

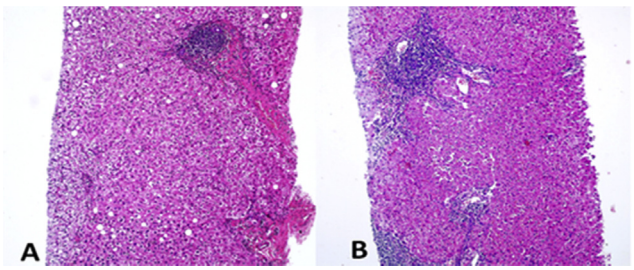


Figure 2. Liver biopsy with METAVIR fibrosis score 2 of (A) chronic HCV-*Toxoplasma* seronegative patient showing portal lymphocytic infiltrate with moderate piece meal necrosis and mild lobular necrosis (activity score 2); (B) chronic HCV-*Toxoplasma* seropositive patient showing portal lymphocytic infiltrate with moderate piece meal necrosis and moderate lobular necrosis (activity score 3).
H & E ×100.

Albumin and platelets count were the laboratory parameters significantly decreased, while INR was significantly increased in *Toxoplasma* positive compared to *Toxoplasma* negative chronic HCV cases, (P = 0.001, 0.04, 0.03, respectively). On the other hand, there was no association between *Toxoplasma* serostatus and levels of bilirubin, ALT, AST, ALP, HB, WBC count and HCV RNA (P = 0.5, 0.3, 0.3, 0.06, 0.2, 0.6, 0.5, Table 4). When Child-Pugh scoring system was adopted to assess the prognosis of liver cirrhosis in chronic HCV group (Table 5), the majority of *Toxoplasma* seropositive cases were class C (13/25, 52%) compared to only 15% of *Toxoplasma* seronegative cases while 36%, 12% of *Toxplasma* seropositive cases, in contrast to 40%

Table 4

Laboratory parameters in chronic HCV patients according to *Toxoplasma* serostatus.

Parameter	Seropositives	Seronegatives	*P*
Albumin (g/dL)	3.34 ± 0.44	3.81 ± 0.62	0.001*
Bilirubin (mg/dL)	1.50 ± 0.90	1.37 ± 0.80	0.500
ALT (IU/mL)	51.65 ± 12.00	55.20 ± 15.00	0.300
AST (IU/mL)	69.85 ± 21.30	64.81 ± 19.30	0.300
ALP KU	15.03 ± 5.10	12.80 ± 3.80	0.060
INR	1.16 ± 0.16	1.09 ± 0.11	0.030*
Hb (g/dL)	11.12 ± 1.98	11.67 ± 2.10	0.200
WBCs/10^9/L	4.70 ± 1.81	4.53 ± 1.64	0.600
Platelet/10^9/L	129.87 ± 25.00	141.71 ± 22.90	0.040*
HCV PCR × 10^4	201.34 ± 33.57	196.37 ± 31.35	0.500

ALT: alanine aminotransaminase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, KU: king unit, INR: international normalized ratio, Hb: hemoglobin, WBC: white blood cell, HCV: Hepatitis C virus, PCR: Polymerase chain reaction.
*Statistical significance at $P < 0.05$.

Table 5

Child-Pugh score of chronic HCV-related cirrhosis group patients according to *Toxoplasma* serostatus.

Child-Pugh score	Seropositives (n = 25) [n (%)]	Seronegatives (n = 20) [n (%)]	*OR* (95% *CI*)	χ^2	*P*
Child A (r)**	3 (12)	9 (45)			
Child B	9 (36)	8 (40)	0.30 (0.06–1.50)	2.30	0.130
Child C	13 (52)	3 (15)	0.08 (0.01–0.50)	8.86	0.003*

*Statistical significance at $P < 0.05$, **reference group.

and 45% of *Toxoplasma* seronegative cases were class B and A, respectively, with significant statistical difference between Child C and Child A reference group (*OR* = 0.08; 95% *CI*: 0.01–0.5; P = 0.003).

4. Discussion

There have been few attempts to identify the prevalence of toxoplasmosis in patients with hepatic ailment, a medical important point particularly in our region due to the commonness of viral hepatitis. Our study declared a statistically significantly (P = 0.02) higher prevalence of *T. gondii* IgG antibody in patients with liver diseases (59/180, 32.8%) versus control subjects (40/180, 22.2%), a finding that analogues a Turkish study [8], showing high *Toxoplasma* seroprevalence in cirrhotic patients (68.5%) compared to control (48%) and another Egyptian study also showing a 65.5% seroprevalence of *T. gondii* antibodies in patients with acute and chronic hepatic disease against a 27% seroprevalence found in controls [17]. However, a Mexican study [18] demonstrated approximate frequency of anti-*T. gondii* IgG antibodies in liver disease patients (13.3%) and controls (10.7%), possibly because of small study sample size. Yet, levels of anti-*T. gondii* IgG antibodies were comparable in our patient and control groups (P = 0.5), indicating that *T. gondii* infection is not likely to virtually engage in the etiology of liver disease in our patients (data not shown).

The sociodemographic characteristics showed no association to *Toxoplasma* seropositivity in patient groups as the protozoan

is distinguished by many infective stages and variable means of transmission. Among clinical data, lymphadenopathy, history of blood transfusion and reflex impairment were significantly higher in seropositive patients (P = 0.01, 0.0003, 0.0003, respectively). Lymphadenopathy is the most characteristic clinical presentation of toxoplasmosis [10] that accounts for about 15%–20% of unexplained lymphadenopathy cases, especially those affecting cervical nodes [19]. It may occur at different times after the initial *T. gondii* infection, persist, and/or recur for various times independently of the specific antiparasitic treatment [20]. However, fine-needle aspiration cytology is essential to diagnose *Toxoplasma* lymphadenitis [21] especially in immunocompromised patient, as serological analysis can yield false negative result [19].

Toxoplasma infection can be transmitted through blood transfusion [22]. The rate of *Toxoplasma* infection in healthy blood donors varies in different areas of the world depending on the rate of infection in the community. In some areas like Northeast Brazil, North Egypt and North India, more than 50% of blood donors were seropositive for *Toxoplasma* infection [23–25]. To prevent transfusion transmitted toxoplasmosis, at least the patients with a higher risk of clinical consequences of *Toxoplasma* infections should receive *T. gondii* free blood. The approaches could be maintaining an inventory of *T. gondii* IgM negative blood [26] and/or provision ofleucocyte reduced blood components [27].

We noticed that 35.6% of *Toxoplasma* seropositive patients (21/59) have reflex impairment compared to only 12.4% of *Toxoplasma* seronegatives (15/121), a finding that goes in harmony with Alvarado-Esquivel [18]. In human, toxoplasmosis can cause myalgia and muscle weakness due to myositis [28]. Behan [29] postulated that an immune disturbance possibly activates latent infection and induces inflammatory myopathy. Recently, Cuomo [30] reported a case of polymyositis in an immunocompetent patient attributed to toxoplasmosis. Despite the possible implication of the host immune response in pathogenesis of *Toxoplasma* polymyositis [31], the patient didn't respond to corticosteroids but to specific treatment with pyrimethamine and sulfodiazine.

Noteworthy, chronic HCV patients in the present study as well as those with related cirrhosis expressed a higher prevalence of *T. gondii* seropositivity (33.3% and 55.6%, respectively) than patients suffering from chronic HBV and related cirrhosis (11.1% and 20.8%, respectively) with significant statistical difference for each comparison (P = 0.013, 0.006, respectively). A larger sample size, would have better expressed the prevalence of the parasite in chronic HBV patients as adult-onset HBV infections are typically self-limited and cleared in about 95% of patients [32]. Only about one-third of adults develop jaundice and hepatitis and less than 1% presents a fulminant course [33]. In contrast, only a minority (about 30%) of HCV-infected adults is able to clear the virus spontaneously [34].

Moreover, the results in Table 3 shows the higher prevalence of *Toxoplasma* seropositivity found in chronic HCV patients with late stages fibrosis (stages F3 and F4) as compared to early stages (stages F1 and F2) besides the significant P value of 0.0008 when the former was compared to F0 stage. Worth mentioning, sampling error [35] and inter-and-intra observer variability [36] are considerable contributing factors [37]. Seropositivity of *T. gondii* was also found to be significantly increased with the higher activity of inflammation (P = 0.01) and associated advanced degree and extent of necrosis. It is possible that *Toxoplasma*, which is known to cause partial damage to the liver, may have a turn in the commencement and clinical course of cirrhosis. *T. gondii* influence on the hepatic disease evolution and vice versa is not clear. Nevertheless, the observed high vulnerability to *T. gondii* infection among hepatic patients demonstrates the existence of a correlation. This observation is not surprising since cirrhosis results in immunodeficiency state which favors *T. gondii* infection.

Immunity to toxoplasmosis is largely T-cell mediated; $CD8^+$ T cells play a major role as effector lymphocytes against the parasite [38], whereas $CD4^+$ T cells are important to regulate immune responses to *T. gondii* [39] and both are known to act synergistically providing a protective immunity that allows the survival of the host during chronic infection [40]. IFN-γ is critical for mediating protective immunity to *Toxoplasma*, and during chronic infection $CD8^+$ T cells are a major source of this cytokine, which is essential for controlling parasite reactivation [41]. Comparably, spontaneous viral clearance of HBV and HCV infection demands vigorous and sustained multi-epitope-specific $CD4^+$ and $CD8^+$ T-cell responses during the acute phase of infection. In contrast, late, transient, week or narrowly focused $CD4^+$ and $CD8^+$ T-cell responses depict chronic infection with both viruses [42,43].

The laboratory parameters in chronic HCV group were within or slightly altered than normal values, a finding that supports the biological role of regulatory $CD4^+$ T cells [44], though albumin and platelets count were significantly lower and INR was significantly higher in *Toxoplasma* positive compared to *Toxoplasma* negative chronic HCV patients (P = 0.001, 0.04, 0.03, respectively). Several factors, including parasitic and infectious diseases, may influence the fluctuation of serum proteins. Da Silva [45] and Bottari [46] reported decreased level of serum albumin in experimentally *Toxoplasma* infected mice due to liver injury.

Theoretically, any bacterial or protozoal infection can be associated with thrombocytopenia that is caused by mechanisms such as increased clearance of damaged platelets with endotoxins, exotoxins, or platelet-activating factor or direct platelet toxicity caused by the microorganism, immune-mediated destruction of the platelets, and platelet adherence to damaged vascular surfaces [47]. Rarely, acquired toxoplasmosis in an immunocompetent patient may be associated with severe thrombocytopenia [48], but thrombocytopenia was noted in congenital toxoplasmosis, in six of seven parasitologically proved cases [49] and in experimentally infected rats, following infection with tachyzoites [50]. The exact pathogenesis is not clear. Of interest, blood platelets are suggested to have a role in destroying parasites [51]. In toxoplasmosis, a platelet derived growth factor isolated from α-granules of platelets, elicited a human platelet-mediated cytoinhibition of *T. gondii* intracellular growth in vitro, in absence of antibodies [50]. Its action against intracellular tachyzoites also includes increased IL-6 secretion that is one of the most potent enhancers of NK cell production of IL-17, which is essential for generating an optimal polymorphonuclear response against *T. gondii* infection [52,53]. Consequently, possibly thrombocytopenia in hepatic patients renders them more susceptible to toxoplasmosis.

Though being within normal range, higher INR was found in *Toxoplasma* positive-chronic HCV patients, mostly because of longer prothrombin time (PT). The importance of the last two findings gathered is highlighted by the concept of Bonacini [54] who declared that thrombocytopenia and prolonged PT are routine test results that predict likelihood of cirrhosis. In general, the liver, as the site of HBV and HCV infection is known to be a tolerogenic environment. For example, murine Kupffer cells constitutively express the immunosuppressive cytokines IL-10 and transforming growth factor β that are involved in the generation of a unique cytokine environment mainly inducing tolerance of liver-infiltrating lymphocytes [55].

Child's score, initially termed Child-Turcotte score [56], or the modified version termed Child-Pugh score [57] is used to assess the prognosis of chronic liver disease, mainly cirrhosis. It includes two continuous variables (bilirubin and albumin) and three discrete variables (ascites, encephalopathy, and PT) which were empirically selected because of their own influence on the prognosis in this context [58]. Applying the score assessment to chronic HCV-related cirrhosis patients in our study obviated that the majority of *Toxoplasma* seropositive cases were class C (52%) compared to only 15% of *Toxoplasma* seronegative patients while 12% of *Toxplasma* seropositive cases, opposed to 45% of *Toxoplasma* seronegative patients were class A, with significant statistical difference between Child C and Child A reference group (P = 0.003).

Severe lesions due to acute toxoplasmosis have been noticed in visceral organs such as the liver, lung, and spleen of mammalian species [59]. In the liver, it causes pathological changes that headway to hepatomegaly, granuloma, hepatitis, and necrosis [60]. Moreover, some epidemiological studies have reported an association of *T. gondii* infection with liver cirrhosis [18]. Intravital microscopy imaging studies have determined that apicomplexan parasites such as *T. gondii* invade the liver, gradually move toward the surface of sinusoidal epithelial cells to the Kupffer cells, ultimately entering and developing in the cytoplasm of hepatocytes [61]. Experimentally, Atmaca [60] found parasite clusters became apparent in both hepatocytes and stellate cells 4 and 6 d after infection and reported that the number of activated hepatic stellate cells (HSCs); known to play an important role in the development of fibrosis and its advancement to cirrhosis [62] was significantly higher in the *T. gondii* infected group than that in the healthy group. Besides, HCV-infected hepatocytes release transforming growth factor β and other profibrogenic factors that differentially modulate HSC expression of several key genes involved in liver fibrosis [63] documenting the role of HSCs in HCV-mediated liver fibrosis.

From the fore mentioned findings, it is plausible to conclude that patients suffering from viral hepatitis particularly HCV are vulnerable to toxoplasmosis as evidenced by *Toxoplasma* seropositivity. Consequent evolution of hepatic pathology and development of cirrhosis must be considered. More detailed studies are needed to address this matter particularly in Egypt wherein one of the highest HCV prevalence in the world exists. Because the majority of the mortality caused by *Toxoplasma* is due to parasite reactivation in immunocompromised patients, there is an urgent need for development of immunotherapeutic vaccination approaches to combat this infection in hepatic patients whom form a risk group for *Toxoplasma*.

Acknowledgments

We gratefully acknowledge Dr. Azza Abdel-Aziz for the analysis of histopathology data and helpful discussions. We also thank the nurses and technicians of Tropical Medicine Department for their excellent assistance. Laboratory work was performed using the logistics of the Center for Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt.

References

[1] Boughattas S, Ayari Kh, Aoun K, Bouratbine A. Survey of the parasite *Toxoplasma gondii* in human consumed ovine meat in Tunis city. *PloS One* 2014; **9**(1): e85044.
[2] Zhou P, Chen Z, Li H, Zheng H, He S, Lin R, et al. *Toxoplasma gondii* infection in humans in China. *Parasite Vectors* 2011; **4**: 165.
[3] Amichay D, Gazzinelli RT, Karupiah G, Moench T, Sher A, Farber J. The genes for the chemokines MuMig and Crg-2 are induced in protozoan and viral infections in response to IFN-γ with patterns of tissue expression that suggest non-redundant roles in vivo. *J Immunol* 1996; **157**: 4511-4520.
[4] Tandon P, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. *Semin Liver Dis* 2008; **28**: 26-42.
[5] Weitberg AB, Alper JC, Diamond I, Fligiel Z. Acute granulomatous hepatitis in the course of acquired toxoplasmosis. *New Eng J Med* 1979; **300**: 1093-1096.
[6] Ortego T, Robey B, Morrison D, Chan C. Toxoplasmic chorioretinitis and hepatic granulomas. *Am J Gastroenterol* 1990; **85**(10): 1418-1420.
[7] Tiwari I, Rolland CF, Popple AW. Cholestatic jaundice due to *Toxoplasma* hepatitis. *Postgrad Med J* 1982; **58**: 299-300.
[8] Ustun S, Aksoy U, Dagci H, Ersoz G. Incidence of toxoplasmosis in patients with cirrhosis. *World J Gastroenterol* 2004; **10**: 452-454.
[9] Wendum DN, Carbonell M, Svrcek M, Chazouillères O, Fléjou JF. Fatal disseminated toxoplasmosis in a *Toxoplasma* seropositive liver transplant recipient. *J Clin Pathol* 2002; **55**(8): 637.
[10] Ho-Yen DO. Clinical features. In: Ho-Yen DO, Joss AWL, editors. *Human toxoplasmosis*. Oxford: Oxford University Press; 1992, p. 56-78.
[11] Schmidt J, Blum HE, Thimme R. T-cell responses in hepatitis B and C virus infection: similarities and differences. *Emerg Microbes Infect* 2013; **2**(3): e15.
[12] Jankovic D, Liu Z, Gause WC. Th1- and Th2-cell commitment during infectious disease: asymmetry in divergent pathways. *Trends Immunol* 2001; **22**(8): 450-457.
[13] Supali T, Verweij JJ, Wiria AE, Djuardi Y, Hamid F, Kaisar MM, et al. Polyparasitism and its impact on the immune system. *Int J Parasitol* 2010; **40**(10): 1171-1176.
[14] Colloredo G, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. *J Hepatol* 2003; **39**(2): 239-254.
[15] Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293.
[16] Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474.
[17] Ghanam ME, Shataat MA, Mel-S Monib, Hassan AA, Younis AI. Evaluation of the role of some parasitic infections as a cause of acute and chronic hepatic diseases. *J Egypt Soc Parasitol* 2001; **31**(1): 37-42.
[18] Alvarado-Esquivel C, Torres-Berumen JL, Estrada-Martínez S, Liesenfeld O, Mercado-Suarez MF. *Toxoplasma gondii* infection and liver disease: a case–control study in a Northern Mexican Population. *Parasit Vectors* 2011; **4**: 75; http://dx.doi.org/10.1186/1756-3305-4-75.

[19] Chen X, Remotti F, Tong GX, Gorczyca E, Hamele BD. Fine needle aspiration cytology of subcutaneous toxoplasmosis: a case report. *Diagn Cytopathol* 2010; **38**(10): 716-720.
[20] Paul M. Immunoglobulin G avidity in diagnosis of Toxoplasmic Lymphadenopathy and Ocular Toxoplasmosis. *Clin Diagn Lab Immunol* 1999; **6**(4): 514-528.
[21] Hosokawa S, Kusama Y, Ono T, Mineta H. *Toxoplasma* lymphadenitis diagnosed by fine-needle aspiration cytology: a rare finding. *J Laryngol Otol* 2014; **128**(6): 561-564.
[22] Singh G, Sehgal R. Transfusion-transmitted parasitic infections. *Asian J Transfus Sci* 2010; **4**(2): 73-77.
[23] Coêlho RA, Kobayashi M, Carvalho LB Jr. Prevalence of IgG antibodies specific to *Toxoplasma gondii* among blood donors in Recife, Northeast Brazil. *Rev Inst Med Trop Sao Paulo* 2003; **45**(4): 229-231.
[24] Elsheikha HM, Azab MS, Abousamra NK, Rahbar MH, Elghannam DM, Raafat D. Seroprevalence of and risk factors for *Toxoplasma gondii* antibodies among asymptomatic blood donors in Egypt. *Parasitol Res* 2009; **104**(6): 1471-1476.
[25] Elhence P, Agarwal P, Prasad KN, Chaudhary RK. Seroprevalence of *Toxoplasma gondii* antibodies in North Indian blood donors: implications for transfusion transmissible toxoplasmosis. *Transfus Apher Sci* 2010; **43**(1): 37-40.
[26] McDonald CP, Barbara JA, Contreras M, Brown S. Provision of a panel of anti-*Toxoplasma*-negative blood donors. *Vox Sang* 1989; **57**(1): 55-58.
[27] Wendel S. Current concepts on transmission of bacteria and parasites by blood components. *Sao Paulo Med J* 1995; **113**(6): 1036-1052.
[28] Plonquet A, Bassez G, Authier FJ, Dray JM, Farcet JP, Gherardi RK. Toxoplasmic myositis as a presenting manifestation of idiopathic CD4 lymphocytopenia. *Muscle Nerve* 2003; **27**(6): 761-765.
[29] Behan WM, Behan PO, Draper IT, Williams H. Does *Toxoplasma* causepolymyositis? Report of a case of polymyositis associated with toxoplasmosis and a critical review of the literature. *Acta Neuropatol* 1983; **61**(3–4): 246-252.
[30] Cuomo G, D'Abrosca V, Rizzo V, Nardiello S, La Montagna G, Gaeta GB, et al. Severe polymyositis due to *Toxoplasma gondii* in an adult immunocompetent patient: a case report and review of the literature. *Infection* 2013; **41**(4): 859-862.
[31] Shapira Y, Agmon-Levin N, Selmi C, Petríková J, Barzilai O, Ram M, et al. Prevalence of anti-*Toxoplasma* antibodies in patients with autoimmune diseases. *J Autoimmun* 2012; **39**(1–2): 112-116.
[32] Bauer T, Sprinzl M, Protzer U. Immune control of hepatitis B virus. *Dig Dis* 2011; **29**(4): 423-433.
[33] Aspinall EJ, Hawkins G, Fraser A, Hutchinson SJ, Goldberg D. Hepatitis B prevention, diagnosis, treatment and care: a review. *Occup Med (Lond)* 2011; **61**(8): 531-540.
[34] Klenerman P, Thimme R. T cell responses in hepatitis C: the good, the bad and the unconventional. *Gut* 2012; **61**: 1226-1234.
[35] Hübscher SG. Histological grading and staging in chronic hepatitis: clinical applications and problems. *J Hepatol* 1998; **29**(6): 1015-1022.
[36] Goldin RD, Goldin JG, Burt AD, Dhillon PA, Hubscher S, Wyatt J, et al. Intra-observer and inter-observer variation in the histopathological assessment of chronic viral hepatitis. *J Hepatol* 1996; **25**(5): 649-654.
[37] Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2014; **20**(2): 475-485.
[38] Suzuki Y, Orellana MA, Schreiber RD, Remington JS. Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science* 1988; **240**: 516-518.
[39] Gazzinelli RT, Hakim FT, Hieny S, Shearer GM, Sher A. Synergistic role of $CD4^+$ and $CD8^+$ T lymphocytes in IFN-gamma production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. *J Immunol* 1991; **146**(1): 286-292.
[40] Pfaff AW, Abou-Bacar A, Letscher-Bru V, Villard O, Senegas A, Mousli M, et al. Cellular and molecular physiopathology of congenital toxoplasmosis: the dual role of IFN-γ. *Parasitology* 2007; **134**: 1895-1902.
[41] Suzuki Y. Host resistance in the brain against *Toxoplasma gondii*. *J Infect Dis* 2002; **185**(S1): S58-S65.
[42] Chisari FV, Isogawa M, Wieland SF. Pathogenesis of hepatitis B virus infection. *Pathol Biol (Paris)* 2010; **58**(4): 258-266.
[43] Schulze Zur Wiesch J, Ciuffreda D, Lewis-Ximenez L, Kasprowicz V, Nolan BE, Streeck H, et al. Broadly directed virus specific $CD4^+$ T cell responses are primed during acute hepatitis C infection, but rapidly disappear from human blood with viral persistence. *J Exp Med* 2012; **209**(1): 61-75.
[44] Bolacchi F, Sinistro A, Ciaprini C, Demin M, Capozzi M, Carducci FC, et al. Increased hepatitis C virus (HCV)-specific $CD4^+CD25^+$ regulatory T lymphocytes and reduced HCV-specific $CD4^+$ T cell response in HCV-infected patients with normal versus abnormal alanine amino transferase levels. *Clin Exp Immunol* 2006; **144**(2): 188-196.
[45] Da Silva AS, Tonin AA, Thorstenberg ML, Leal DB, Fighera R, Flores MM, et al. Relationship between butyrylcholinesterase activity and liver injury in mice acute infected with. *Toxoplasma Gondii Pathol Res Pract* 2013; **209**(2): 95-98.
[46] Bottari NB, Tonin AA, Fighera R, Flores MM, França RT, Camillo G, et al. *Neosporacaninum* and *Toxoplasma gondii*: relationship between hepatic lesions, cytological and biochemical analysis of the cavitary liquid during the acute phase of the diseases in experimental models. *Exp Parasitol* 2014; **136**: 68-73.
[47] Tate DY, Carlton GT, Johnson D, Sorenson RL, Nesbit M, White J, et al. Immune thrombocytopenia in severe neonatal infections. *J Pediatr* 1981; **98**(3): 449-453.
[48] Beaman M, McCabe RE, Wong S, Remington JS. Toxoplasma gondii. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*, Vol. 2. London: Churchill Livingstone; 1995.
[49] Desmonts G, Daffos F, Forestier F, Capella-Pavlovsky M, Thulliez P, Chartier M. Prenatal diagnosis of congenital toxoplasmosis. *Lancet* 1985; **1**(8427): 500-504.
[50] Chumpitazi BF, Simon J, Polack B, Peyron F, Picot S, Ricard J, et al. Human platelet inhibition of *Toxoplasma gondii* growth. *Clin ExpImmunol* 1998; **111**(2): 325-333.
[51] McMorran BJ, Marshall VM, de Graaf C, Drysdale KE, Shabbar M, Smyth GK, et al. Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. *Science* 2009; **323**(5915): 797-800.
[52] Kelly MN, Kolls JK, Happel K, Schwartzman JD, Schwarzenberger P, Combe C, et al. Interleukin-17/Interleukin-17 receptor-mediated signaling is important for generation of an optimal polymorphonuclear response against *Toxoplasma gondii*. *Infect Immun* 2005; **73**(1): 617-621.
[53] Passos ST, Silver JS, O'Hara AC, Sehy D, Stumhofer JS, Hunter CA. IL-6 promotes NK cell production of IL- 17 during toxoplasmosis. *J Immunol* 2010; **184**: 1776-1783.
[54] Bonacini M, Hadi G, Govindarajan S, Lindsay KL. Utility of a discriminant score for diagnosing advanced fibrosis or cirrhosis in patients with chronic hepatitis C infection. *Am J Gastroenterol* 1997; **92**: 1302-1304.
[55] You Q, Cheng L, Kedl RM, Ju C. Mechanism of T cell tolerance induction by murine hepatic Kupffer cells. *Hepatology* 2008; **48**: 978-990.
[56] Child CG, Turcotte JC. Surgery and portal hypertension. In: Child CG, editor. *The liver and portal hypertension*. Philadelphia: WB Saunders; 1964, p. 50-64.
[57] Pugh RN, Murray-Lyon IM, Dawson JL,Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649.
[58] Durand F, Valla D. Assessment of the prognosis of cirrhosis: Child-Pugh versus MELD. *J Hepatol* 2005; **42**(Suppl. 1): S100-S107.
[59] Weiss LM, Kami K. *Toxoplasma gondii–the model apicomplexan: perspectives and methods*. London: Academic Press; 2007.
[60] Atmaca HT, Gazyagcı AN, Canpolat S, Kul O. Hepatic stellate cells increase in *Toxoplasma gondii* infection in mice. *Parasite Vectors* 2013; **6**: 135; http://dx.doi.org/10.1186/1756-3305-6-135.

[61] Frevert U, Engelmann S, Zougbédé S, Stange J, Ng B, Matuschewski K, et al. Intravital observation of *Plasmodium berghei* sporozoite infection of the liver. *PLoS Biol* 2005; **3**(6): e192.

[62] Rastogi A, Bihari C, Maiwall R, Ahuja A, Sharma MK, Kumar A, et al. Hepatic stellate cells are involved in the pathogenesis of acute-on -chronic liver failure (ACLF). *Virchows Arch* 2012; **461**: 393-398.

[63] Schulze-Krebs A, Preimel D, Popov Y, Bartenschlager R, Lohmann V, Pinzani M, et al. Hepatitis C virus-replicating hepatocytes induce fibrogenic activation of hepatic stellate cells. *Gastroenterol* 2005; **129**(1): 246-248.