



ISSN: 0067-2904

Seroprevalence of Toxoplasmosis in patients with chronic liver disease in Baghdad

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Abstract

The influence of *Toxoplasma gondii* in the pathogenesis of hepatic disease has lately had considerable attention. The objective of this study is to assess the seroprevalence of *T. gondii* infection in patients with chronic liver disease from Baghdad-Iraq. All patients have attended Gastroenterology and Hepatology Teaching Hospital, Medical city in Baghdad, Iraq. An analytical case-control study was achieved from September to November 2018. Seventy male patients with chronic liver disease (CLD) and 70 control males (free of chronic liver diseases) participated in this study, their ages were between (18-80) years old. Serum samples were taken from all subjects and were analysed with enzyme-linked immunosorbent assay (ELISA) for the presence of anti-*T.gondii* IgG and IgM antibodies. The prevalence of anti-*T.gondii* IgG was significantly higher 62.85% in CLD patients compared with 27.28% in the control subjects. Anti-*T. gondii* IgM antibodies were also showed to be significantly higher in CLD patients compared with control subjects. A significant relation between age and the prevalence of *T.gondii* was reported in this study. Toxoplasmosis was largely reported among those aged (>40) years old for both CLD patients and control subjects, while the other age groups showed less seroprevalence rates.

Sera samples for both CLD patients and control subjects were tested for different liver enzyme: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). ALT was greater in CLD-*Toxoplasma* positive patients comparing to CLD-*Toxoplasma* negative patients. In addition to AST was higher in control-*Toxoplasma* positive subjects comparing to control-*Toxoplasma* negative subjects.

These findings show that Toxoplasmosis is high expected to be diagnosed with chronic liver disease patients. Consequently, attention would be focused on health education of peoples at high risk of toxoplasmosis.

Keywords: Liver disease, Liver enzymes, Toxoplasmosis, Iraq.

الانتشار المصلي لداء المقوسات في المرضى المصابين بأمراض الكبد المزمنة في مدينة بغداد

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الخلاصة

زاد الاهتمام مؤخرا بالدور الذي يلعبه طفيلي داء المقوسات لدى الاشخاص المصابين بامراض الكبد المزمنة. يهدف هذا البحث الى تقييم الانتشار المصلي لداء المقوسات في المرضى المصابين بامراض الكبد

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المزمنه في مدينه بغداد. اجريت الدراسة في الفترة ما بين ايلول الى تشرين الثاني من العام 2018، من مستشفى امراض الجهاز الهضمي و الكبد التعليمي، دائرة مدينة الطب حيث تم شمول 70 من الذكور المصابين بامراض الكبد المزمنه و 70 من الذكور غير المصابين بامراض الكبد المزمنه (مجموعة السيطرة) تراوحت اعمار جميع المشمولين بالبحث بين (18-80) سنة. جمعت عينات مصل الدم منهم جميعا وفحصت بتقنية الامتزاز المناعي المرتبط بالانزيم للتحري عن الاضداد المناعية المضادة للمقوسات الكوندية نوع IgG و IgM. اظهرت النتائج ان الانتشار المصلي للاضداد المناعية المضادة للمقوسات الكوندية نوع IgG كان عاليا (62.85%) و بشكل معنوي في المرضى المصابين بامراض الكبد المزمنه مقارنة بانتشار مصلي اقل (27.28%) لدى مجموعة السيطرة. كما اظهرت الاضداد المناعية المضادة للمقوسات الكوندية نوع IgM نسبة انتشار اعلى لدى المرضى المصابين بامراض الكبد المزمنه و بفرق معنوي واضح مقارنة بمجموعة السيطرة. سجل في هذا البحث ايضا نسبة عالية من داء المقوسات ممن هم بعمر (<40) سنة لكل من المرضى المصابين بامراض الكبد المزمنه و مجموعة السيطرة.

تضمنت الدراسة ايضا فحص فعالية انزيمات اسبارتيت الناقل لمجموعة الامين AST ، الالانين الناقل لمجموعة الامين ALT و الفوسفاتيز القاعدي ALP في جميع عينات المرضى و السيطرة. ظهر انزيم الالانين الناقل لمجموعة الامين بمستويات اعلى لدى مرضى الكبد الموجبين للفحص المصلي لطفيلي التوكسوبلازما مقارنة بمرضى الكبد السالبيين لفحص التوكسوبلازما. في حين ظهر انزيم اسبارتيت الناقل لمجموعة الامين مستويات مرتفعة لدى مجموعة السيطرة الموجبة لفحص التوكسوبلازما مقارنة بمجموعة السيطرة السالبة لطفيلي التوكسوبلازما.

نتائج هذه البحث تشير الى ان داء المقوسات يمكن ان يكون متوقعا بصورة اكبر لدى المرضى المصابين بامراض الكبد المزمنه. وعليه فان الجهود يجب ان تتركز على التعليم و التقيف الصحي لدى الاشخاص الاكثر خطرا للتعرض لداء المقوسات.

Introduction

Toxoplasmosis is an infection caused by *Toxoplasma gondii*, a member of the Apicomplexa phylum. Toxoplasmosis is a cosmopolitan infection of humans and other warm-blooded animals. This protozoan parasite is a pathogen of public health and economic concern [1]. Infections initiated by *T. gondii* remain to inflict important public health difficulties since it can cause different clinical aspects such as retino-choroiditis, hydrocephaly, mental retardation, and even death in fetus and life-threatening encephalitis in individuals with AIDS, organ transplant recipients, or receiving immunosuppressive therapy [2, 3]. Globally, it was estimated that between 30% and 65% of all people worldwide are infected with *T. gondii*. Seroprevalence of *T. gondii* infection in human increases with age but it does not differ significantly between sexes, and is lower in cold regions, hot and arid areas, or at high elevations [4].

Though *T. gondii* has neurotropic and ocular affinities, this parasite can also infect additional organs, such as liver, pancreas, spleen, heart, and lymph nodes [5]. Some comparatively small researches have noticed link between *T. gondii* infection and various aspects of liver pathologies, such as hepatomegaly, granuloma, hepatitis, necrosis, jaundice, and cirrhosis [6– 10]. *T. gondii* infection has also been associated to irregular liver function, it can affect the functions of liver enzymes [11, 12]. Recently, concern in evaluating the association between *T. gondii* infection and liver disease has amplified [13, 14], possibly because of the massive worldwide burden and public health impacts of both toxoplasmosis [15] and liver diseases [16]. As significant results exist relating Toxoplasmosis to liver impairment, and there are no existing information about the correlation between toxoplasmosis and liver diseases in Iraq. It is important to recognize some approval connecting *T. gondii* infection to liver disease in Iraq. So, the current study was conducted to assess the seroprevalence of *T. gondii* infection in patients suffered from chronic liver disease from Baghdad-Iraq using enzyme-linked immunosorbent assay (ELISA).

Materials and Methods

Study design subject

This research is an analytical case control study conducted from September to November 2018. The population in this study was adult male patients with chronic liver disease and adult male healthy control subjects. This study was designed to evaluate the relationship of chronic liver disease with *T.*

gondii infection. Seventy outpatients attended in Gastroenterology and Hepatology Teaching Hospital, Medical city in Baghdad, Iraq participated in this research. All patients were diagnosed as chronic liver disease patients (CLD) based on some clinical and biochemical examinations which were done by GIT consultant. The mean age of the CLD patients was 50.11 ± 14.72 years (range: 18-80 years). All patients were from Baghdad except some from other governorates, Diyala (n=3), Anbar (n=1), Babil (n=1) and Al Muthanna (n=1). The causes of liver disease was varied among the CLD patients, the majority was alcohol consumption (n= 29), hepatitis C virus (n=15), Wilson disease (n=2) and unknown cause (n=28).

Seventy control individuals (no chronic liver diseases) were also involved in the study. The mean age in control subjects was 51.97 ± 10.87 (range: 18-71). The healthy control subjects were obtained from the general population of Baghdad, Iraq. This study protocol was approved by Ministry of Health and Environment, Baghdad, Iraq. A permission from all patients and healthy control subjects were also approved.

Detection of the anti-*T. gondii* IgG and anti-*T. gondii* IgM

Serum samples of both CLD patients and control subjects were taken and reserved frozen at -20°C until analysed. They were analysed for anti-*T. gondii* IgG antibodies with the enzyme immunoassay kit “*Toxoplasma* IgG” (Humman). Anti-*T. gondii* IgG antibody levels were stated as International Units (IU)/ml, and a result equivalent or greater than 8 IU/ml was considered positive. In addition, sera positive for anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* IgM antibodies by the commercially available enzyme immunoassay “*Toxoplasma* IgM” kit (Humman). All tests were achieved after the directions of the manufacturer.

Biochemical tests

Sera samples for both CLD patients and control subjects were analysed for different liver enzymes using kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Randox Laboratories Ltd., Antrim, UK), and alkaline phosphatase (ALP) (BioMerieux, Lyon, France) according to the manufacturer’s instructions.

Child-Pugh calculation

Child-Pugh was used to evaluate the prognosis of chronic liver disease and cirrhosis and its relation with toxoplasmosis. Child-pugh was calculated by a calculator found in <https://www.mdcalc.com/child-pugh-score-cirrhosis-mortality>.

Statistical Analysis

The results were stated as percentage and mean \pm standard deviation (SD). Data analysis was achieved by SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The data were assessed by chi-square test and the Student’s t-test (t). P values < 0.05 were considered statistically significant.

Results

Results showed that the Anti-*T. gondii* IgG antibodies were significantly higher in CLD patients comparing with control subjects. Anti-*T. gondii* IgG antibodies were found in 44 (62.85%) of 70 CLD patients and in 17 (24.28%) of 70 controls ($P = 0.00000418$). Of the 44 anti-*T. gondii* IgG positive patients, 22 (50%) had IgG levels higher than 100 IU/ml, and 22(50%) between 7 to 99 IU/ml. In comparison, of the 17 anti-*T. gondii* IgG positive controls, 6(35.29%) had IgG levels greater than 100 IU/ml and 11 (64%) between 7 to 99 IU/ml. Anti-*T. gondii* IgM antibodies were also found significantly higher in CLD patient comparing with control subjects. Anti-*T. gondii* IgM antibodies were detected in 35 (50%) of 70 patients and in 7(10%) of 70 control subjects ($P = 0.00000024$).

Child-Pugh scoring system was calculated to evaluate the prognosis of liver cirrhosis in CLD patients. The majority of *Toxoplasma* seropositive - CLD patients were reported in class-C (Table-1), although no significant relation ($p > 0.05$) was noticed between the child-Pugh classes and the seroprevalence of *T.gondii*.

Table-2 illustrates the seroprevalence based on patients’ age. CLD patients showed high seroprevalence rate of *T.gondii* in all age groups. The highest seroprevalence of *T. gondii* infection was 88.2% which reported in CLD patients whose ages were less than 40 years, while the other age groups showed less seroprevalence rates (Table-2). The statistical analysis showed significant relation between age and seroprevalence of *T.gondii* in both CLD patients and control subjects ($p < 0.05$).

The results of liver enzymes for both CLD patients and control group are illustrated in Table-3. Results showed a non-significant participated ($p > 0.05$) of the liver enzymes: AST, ALT, and ALP in the sera of positive *T.gondii* and negative patients. Although ALT was grater in CLD-*T.gondii* positive

patients comparing to CLD-*T.gondii* negative patients. As well as AST was higher in control-*Toxoplasma* positive subjects comparing to control-*Toxoplasma* negative subjects.

Table 1-Prevalence of *Toxoplasma gondii* related to age for CLD patients and Control group

Age group/years	Cases with liver diseases			Control group			P-value
	No. of tested	No. of positive	Prevalence of <i>T.gondii</i> (%)	No. of tested	No. of positive	Prevalence of <i>T.gondii</i> (%)	
>40	17	15	88.2	9	3	33.3	0.0039
41-50	17	10	58.8	22	6	27.27	0.011
51-60	16	8	50	22	4	18.18	0.037
<60	20	11	55	17	4	23.52	0.051

Table 2-Child –Pugh score of CLD patients according to *Toxoplasma gondii* seroprevalence

Child-Pugh Class	CLD patients	
	<i>Toxoplasma</i> + ve n(%) n=45	<i>Toxoplasma</i> –ve n(%) n=25
Child B	22(48.8%)	11 (44%)
Child C	23 (51.11%)	14 (56%)

Table 3-Effects *Toxoplasma gondii* infection on the levels of liver enzymes in chronic liver disease patients and control subjects.

Liver Enzymes	CLD patients		Control	
	<i>Toxoplasma</i> + ve	<i>Toxoplasma</i> –ve	<i>Toxoplasma</i> + ve	<i>Toxoplasma</i> – ve
ALP IU/L (95% CI)	176.7 (127.9- 225.5)	185 (119.9-250.8)	90 (74.47- 105.4)	91.6 (82.84-100.4)
ALT IU/L (95% CI)	51.62 (41.34-61.9)	40.12 (26.33-53.9)	39 (33.74-47.79)	43.66 (39.68-47.64)
AST IU/L (95% CI)	67.66 (55.54-80.01)	69.32 (52.91-85.73)	28.2 (24.96-31.39)	26.9 (25.12-2876)

Discussion

Toxoplasma gondii infections exhibits variable clinical aspects. Assessment of its prevalence in some risk groups in the community and founding of these risk groups act an important part in preparing the required precautions against this protozoal infection[17].In the current study *Toxoplasma* ELISA antibody positivity was significantly greater in chronic liver disease patients. A higher incidence of anti-*T. gondii* IgG and IgM antibodies was reported in CLD patients than control group. Studies regarding the link of *T. gondii* infection in liver disease patients in Iraq are very limited. The high level of anti-*T. gondii* IgM and IgG levels in CLD patients than in control group may be explained by the declining of cell-mediated and humoral immune response in chronically infected patients due to subsequent reactivation of latent infection[18, 19]. Our results agrees with that stated by Ghanam *et al* [20]who reported high seroprevalence (65.5%) of *T. gondii* antibodies in patients with acute and chronic liver diseases against a 27% seroprevalence found in the group of healthy control subjects. Our results also agreed with other investigators who stated an association of Toxoplasmosis with liver diseases [21, 22]. On other hand our results disagreed with those illustrated by Alvarado-Esquivel, *et al.* [23]who did not show any link between seropositivity to *T. gondii* and liver diseases with comparable seroprevalence of *T. gondii* IgM and IgG levels in CLD patients and control subjects. Undoubtedly, differences in the features of the studies could clarify the dissimilarities in the seroprevalences including the use of dissimilar laboratory approaches, difference in sex and ages of participants and sample size of CLD patients and control subjects. In this study the majority of

Toxoplasma seropositive - CLD patients were reported in class C this result agreed with the findings of El-Henawy *et al.* [24] who presented that the majority of *Toxoplasma* seropositive cases were class C.

Toxoplasmosis was largely reported among those aged (>40) years old for both CLD patients and control subjects, while the other age groups showed less seroprevalence rates. The reduction in seroprevalence with age is likely to be a function of the development of immunity induced by previous infections [25].

Liver enzyme activities are good indicators of liver cell injury. Generally, these enzymes are found in the liver and other tissues where they function in energy metabolism involving the transamination of amino acids. Nevertheless, in cases of impairment in liver cells, AST and ALT might drop out into the general circulation leading to elevated activity [26]. Liver injury is a well-established complication of acute *T.gondii* infections, as this infection can cause cell infiltration especially in the portal areas[27]. Furthermore, protein fractions of ALT and AST diverse according to the strength of inflammation provoked by *T.gondii* infection. In the current study, ALT was higher in CLD-*Toxoplasma* positive patients comparing to CLD-*Toxoplasma* negative patients. In addition to AST was higher in control-*Toxoplasma* positive subjects comparing to control-*Toxoplasma* negative subjects. These results agreed with the results of Limdi and Hyde [28] and Mahmood and Dawood [29]. This results might be described by the presence of *T. gondii* in some liver tissue parts like the cells of bile duct, meanwhile hepatic ALP is known to be found on the canalicular and luminal portions on the bile duct epithelial tissue [28]. From the previously mentioned results, it is likely to conclude that patients suffering from chronic liver diseases are susceptible to toxoplasmosis as showed by *Toxoplasma* seropositivity. Consequent evolution of liver pathology and development of some complications such as cirrhosis must be measured. More comprehensive studies are needed to address this issue in Iraq.

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