

Liver function tests in toxoplasmosis

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ABSTRACT

Objective: To highlight the possible effects of toxoplasmosis on serum activities of liver enzymes.

Methods: Sixty serologically toxoplasmosis positive women referred to the Public Health Laboratory Centre in Nineveh Province were enrolled during the period from Oct. 2008 – March 2009. Their ages ranged between 16-35 years and mean±SD (24.9±4.8 years). Cases were compared with 40 age matched apparently healthy high school, medical college students and employees of Mosul College of Medicine control women who were serologically negative for toxoplasmosis; their ages ranged between 17-35 years and mean ± SD (23.9± 5.5years).

Serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and lactate dehydrogenase (LDH) were measured in all cases and controls.

Results: Liver enzymes activities were higher in patients compared with those of the controls.

Conclusion: A probable involvement of the liver in the disease process was noticed for the toxoplasmosis patients although not sufficient to produce clinical signs and symptoms of liver disease.

Keywords: Liver enzymes, toxoplasmosis.

الخلاصة

تمت دراسة تأثير داء المقوسات على خمائر الكبد في أمصال (٦٠) امرأة من سكان مدينة الموصل بشمال العراق مصابات بداء المقوسات لأول مرة. تم التثبت من التشخيص بواسطة فحصين لمصل الدم: فحص تلازن اللاتكس وفحص ارتباط الخميرة للادمصاص المناعي - الغلوبين المناعي صنف م. المشاركات بهذه الدراسة كن غير حوامل، غير مدخنات ولا يستعملن أي علاج. أعمارهن تراوحت بين (١٦-٣٥) سنة مع معدل ± انحراف معياري (٥.٥ ± ٢٤.٩). شملت الدراسة قياس فعالية الإنزيمات ناقلي الامين الاسبارتيت والالنين، انزيم الفوسفاتيز القاعدي، انزيم ناقل كما كلوتاميل وانزيم لاكتيت نازعة الهيدروجين في مصل الدم. تمت مقارنة النتائج مع ٤٠ امرأة غير مصابة وبكامل الصحة وتتراوح أعمارهن بين (١٧-٣٥) سنة مع معدل ± انحراف معياري (٥.٥ - ٢٣.٩) سنة. أظهرت نتائج المقارنة ان انزيم ناقل الامين الاسبارتيت أعلى عند المصابات بداء المقوسات مقارنة بغير المصابات (ب = ٠.٠٠١) وان انزيم ناقل الامين الالنين أعلى عند المصابات (ب > ٠.٠٠١). كذلك كان انزيم الفوسفاتيز القاعدي أعلى عند المصابات (ب = ٠.٠٤٥) وانزيم ناقل كلوتاميل أعلى عند المصابات (ب = ٠.٠١٣). أما بالنسبة لإنزيم لاكتيت نازعة الهيدروجين فلم يكن هنالك أية زيادة إحصائية لهذه الخميرة عند المصابات (ب < ٠.٠٥). تستنتج الدراسة تأثيرا بسيطا على إنزيمات الكبد في مصل دم المصابات بداء المقوسات وهذه الزيادة لا تكفي لحدوث علامات سريرية لالتهاب الكبد عند المصابات.

الكلمات المفتاحية: داء المقوسات، وظائف الكبد.

Toxoplasmosis is a well known protozoal infection that is caused by the parasite *Toxoplasma gondii* ⁽¹⁾ which infests nearly one –

third of the world human population⁽²⁾. Toxoplasmosis could be either congenital where it is transmitted through the placenta⁽³⁾ or acquired

by several pathways including contact with contaminated food⁽⁴⁾, dust, soil, cats and litter box material.⁽⁵⁾ It can be also acquired via transplantation of infected organs, through blood transfusion and through laboratory accidents⁽⁶⁾.

Many human and animal studies concerned with the involvement of the liver in cases of toxoplasmosis were published as early as 1965⁽⁷⁾. Later, an association between toxoplasmosis with hepatomegally and some abnormal liver function tests was found⁽⁸⁾. Other studies related the development of granulomatous hepatitis to toxoplasmosis⁽⁹⁾. The involvement of the liver in toxoplasmosis with no evidence of symptomatic hepatitis was also attributed to toxoplasmosis⁽¹⁰⁾.

Furthermore, it was reported that cholestatic jaundice may be due to *Toxoplasma gondii* infection⁽¹¹⁾, however, other reports suggested that toxoplasmosis could be associated with abnormal liver function tests, round cell infiltration in the portal areas, cholestasis, swollen endothelial cells and/or focal necrosis of liver cells⁽¹²⁾. Reports of liver dysfunction in recipients in cases of liver and kidney transplantation were attributed to donors with previous history of toxoplasmosis^(13, 14).

Experimental animal studies in parallel with human studies further explained some of the pathological liver changes in toxoplasmosis to be due to increased production of interleukin 12 (IL-12) and tumor necrosis factor-alpha (TNF- α) by the Kupffer cells of the liver⁽¹⁵⁾. Other researchers have confirmed this finding and added more explanations like the increase in serum T1cytokines including interferon-gamma (INF- γ), TNF- α , IL-12 and IL-18. The extent of tissue inflammation is often disproportionate with the presence of parasite suggesting that the pathology is partially immune mediated⁽¹⁶⁾. The Kupffer cells of the liver are part of the immune system in human body and can produce different inflammatory cytokines (TNF- α , IL-12, INF- γ and NO) which may play a role in pathogenesis of liver injury⁽¹⁷⁾.

The aim of the current work is to assess the impact of toxoplasmosis on serum liver enzymes AST, ALT, ALP, GGT and LDH in our locality by comparison with the healthy controls.

SUBJECTS AND METHODS

The study assessed 60 non- pregnant serologically positive cases with toxoplasmosis diagnosed for

the first time referred to the Public Health Center in Nineveh Province. The study was conducted over a period of six months from 1st October 2008 through 31st March 2009; patients' ages ranged between 16-35 years (24.9 \pm 4.8 years). Serological confirmation was by latex agglutination test (LAT) (more than 10 IU/ μ l). This test is considered to be a serological screening test for *Toxoplasma* antibody especially by small laboratories in remote areas due to its availability, simplicity, and sensitivity. It showed 100 % sensitivity but its positive predictive value was only 71.3 %⁽¹⁸⁾. It was followed by Enzyme Linked Immunosorbant Agglutination test (ELISA –IgM). Values of Toxo M index greater than 1.00 were considered positive. A recent study evaluated the sensitivity and specificity of ELISA –IgM; it showed 92% sensitivity and 100% specificity which corresponds to 97.4% negative predictive value and 100% positive predictive value⁽¹⁹⁾. Patients with positive history of jaundice and liver diseases, drug intake, smoking or any clinical abnormality were excluded from the study. Forty apparently healthy control women were enrolled as a control group. They were 17-35 years old with a mean age of (23.9 \pm 5.5 years). They were all negative for both latex and ELISA –IgM tests. LAT and ELISA were performed at the Public Health Lab. Centre in Mosul. Analysis of enzymes was conducted in the Biochemistry Laboratory - Mosul College of Medicine.

Non-hemolysed serum samples were obtained from patients and controls and divided into two aliquots, the first was used for the serological tests and the second was stored in -20C^o for serum enzymes analyses in daily batches. Internal quality controls (IQC) were used within run and batch respectively.

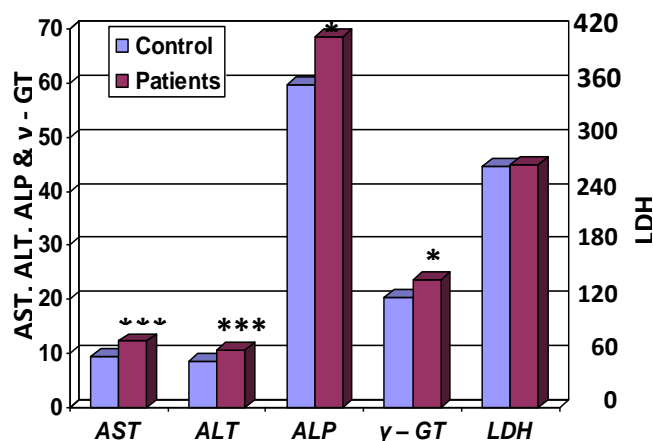
Activities of serum enzymes were measured using kits for AST and ALT method of Reitman & Frankel⁽¹⁸⁾ (Randox, UK), ALP-method of Kind & King⁽¹⁹⁾ (BioMerieux, France), GGT- method of Szasz⁽²⁰⁾ (Biolabo, France) and LDH – method of Henry⁽²¹⁾ (Biolabo, France).

Serological tests for toxoplasmosis as a screening test were applied using (LAT) technique^(22,23) (Biokit, Spain). The semi quantitative LAT was considered positive when the serum contained more than 10 IU/ul of toxoplasma antibodies. ELISA IgM test was done using antigen –antibody

reaction with horse – radish peroxidase forming a conjugate whose reaction is stopped by adding tetramethyl benzidine. The intensity of the colour is preoperational to the amount of IgM⁽²⁴⁾. ELISA test was considered positive for IgM antibody to *Toxoplasma gondii* if the Toxo M index was 1.00 or greater indicating the probability of current or recent toxoplasmosis. For analysis of data, unpaired student Z –test for the comparisons⁽²⁵⁾.

RESULTS

Table 1 and **Figure 1** show the comparison of serum enzymes activities between the patients and control groups. Serum AST and ALT activities were significantly higher in patients compared to the control at ($p \leq 0.001$), also did the activities of ALP and GGT ($p < 0.05$). However, the increment in serum LDH activity was not significant.



* Significant difference at $p \leq 0.05$, *** at $p \leq 0.001$.

Figure 1. Comparison of measured enzymes activities between patients and controls.

Table 1. Comparison of measured serum enzyme activities between patients and controls.

Liver Enzymes	Control (n=40)			Patients (n=60)			p-value
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	
AST (IU/L)	9.57 ± 3.46	9.0	3-18	12.27 ± 3.62	11.5	6.0-21.0	<0.001
ALT (IU/L)	8.45 ± 2.66	8.5	4-18	10.58 ± 3.16	10.0	5.0-20.0	<0.001
ALP (IU/L)	59.57 ± 16.97	57.87	37.63-114.31	68.57 ± 23.22	66.39	22.01-121.4	0.045
γ - GT (IU/L)	20.52 ± 4.72	20.65	10.4-29.5	23.36 ± 5.23	22.7	10.7-34.4	0.013
LDH (IU/L)	267.51 ± 47.0	262.3	197.3-372.4	269.29 ± 43.2	274.4	184.9-356.3	(NS)

NS = Non- significant at $p < 0.05$. Comparisons are by unpaired student's z- test.

DISCUSSION

Serum AST and ALT activities are excellent markers of hepatocellular injury⁽²⁶⁾ and serum ALT activity is more specific than serum AST for assessing liver injury⁽²⁷⁾. The significantly elevated serum activities of aminotransferases in the serologically positive cases of toxoplasmosis in this study are in agreement with several studies⁽²⁸⁻³¹⁾. These results also agree with the studies performed on experimental animals^(32,35-37).

These elevations suggest the involvement of liver cells. Hepatic necrosis is a well established complication of toxoplasmosis⁽³²⁾ where this infection can cause round cell infiltration in the portal areas, cholestasis, swollen endothelial cells and focal necrosis of liver cells⁽¹²⁾. However, despite the significant increase of AST and ALT activities compared with the controls, the levels are still within normal ranges suggesting a mild effect on the liver.

Serum ALP activity was significantly higher in the patients group than that the control ($p=0.045$). This finding is in agreement with that reported by several studies^(31, 32, 38, 39) and could be explained by the presence of *Toxoplasma gondii* parasites in the bile duct cells since hepatic ALP is reported to be present on the canalicular and luminal domain on bile duct epithelium⁽²⁸⁾.

Serum GGT activity was significantly higher in the patients group than that of the control ($p=0.013$) and this is in accordance with previous studies^(31, 32). This enzyme is liberated from intra and extra duct cells and present in hepatocytes⁽⁴⁰⁾. The elevation of this enzyme is more evident than that of serum ALP activity; this may suggest that the bile ducts are involved as well as the hepatocytes in cases of toxoplasmosis. Since GGT is more sensitive than ALP in the diagnosis of obstructive liver disease⁽⁴¹⁾, this will imply that there is definite liver involvement in cases of infected patients. LDH activity showed no

significant change in both groups, this could be explained on the basis that only a fraction of LDH (isoenzyme LD5) is liver specific⁽⁴²⁾ and this seems to be in agreement with some other reports^(36,43,44). It is obvious that the elevation of serum enzymes activities (except LDH) in this study might suggest liver involvement in toxoplasmosis since the parasite has been reported to be present in different organs including the liver^(44,45).

In conclusion, the liver enzymes activities are statistically elevated but they are still within normal acceptable ranges suggesting that toxoplasmosis may affect the liver in a way that this effect is not sufficient to produce clinical signs and symptoms.

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