RESEARCH ARTICLE

Seroprevalence of Toxoplasmosis in Iraqi Patients with Liver Diseases

Rawaa S. Abdullah,* Israa K. AL-Aubaidi

Department of Biology, College of Education (Ibn- AL-Haitham) for Pure Science, University of Baghdad, Baghdad, Iraq

Received: 06th May, 2021; Revised: 17th June, 2021; Accepted: 03rd August, 2021; Available Online: 25th September, 2021

ABSTRACT

Toxoplasmosis is a parasitic protozoan disease that causes a variety of clinical illnesses, most cases are asymptomatic, and some cause severe problems. The present study was aimed to highlight the relationship between toxoplasmosis and different liver diseases, this study consist of 204 samples of patients suffering from liver disease. Tow diagnostic tests were applied Toxo IgG/IgM rapid cassette test and Enzyme-linked immunosorbent assay (ELISA). In addition, some liver enzymes level were measured Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) in serum of study samples. Important epidemiological risk factors were also detected.

Seroprevalence of toxoplasmosis by using Toxo IgG/IgM rapid cassette test were 50 positive cases with liver disease in 24.5% for IgG antibody, and negative cases were 154 (75.49%) with significant differences. All results were negative for IgM antibodies. ELISA test results were Toxoplasma IgG antibody found in 49 cases with 24% percentage in liver disease patient and 8 positive cases were detected for IgM antibody. Significant elevation of some liver enzyme activities was noticed in patients of Liver disease infected with toxoplasmosis compared with the control group. Positive cases in males were 48.71% and 51.28% in females. High rates of seropositive cases were 43.5% in housemaker patients. Toxoplasma seroprevalence was high in HBV patients, 42.30%.

Keywords: IgG, IgM, Liver diseases, Toxoplasmosis.

International Journal of Pharmaceutical Quality Assurance (2021); DOI: 10.25258/ijpqa.12.3.1

How to cite this article: Abdullah RS, AL-Aubaidi IK. Seroprevalence of Toxoplasmosis in Iraqi Patients with Liver Diseases. International Journal of Pharmaceutical Quality Assurance. 2021;12(3):173-178.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite that infects approximately all warm-blooded animals including humans, its considered one of the most effective eukaryotic pathogens.[1] The parasite has a complex life cycle with asexual reproduction occurring in different tissues of the intermediate hosts for example humans and birds and sexual reproduction taking place in digestive epithelium of felines and extreme the final host, Human infect mainly by ingesting food or drink contaminated by oocysts that found in the felids and tissue cysts that found in intermediate hosts.[2] Acute infection occurs in the first few days depending on host immunity with the rapidly growing replication of the tachyzoites. Tachyzoites is converted into bradyzoites (a form similar to intracellular crescent persisting in the tissues of the muscles and brain) over time and formation of tissue cysts in host cells.[3] The inherited contamination can lead to birth defects, including hydroceolalus, spontaneous abortion, chorioretinitis, and intra cerebral calcification.[4]

Liver disease is any disturbance of liver function that causes illness. The liver is in charge of numerous critical functions inside the body when it becomes diseased or injured, the loss of those functions can make noteworthy harm the body. Liver disease is also referred as hepatic disease. Liver disease is a wide term that covers all the potential problems that reason the liver to fail to perform its designated functions. Usually over 75% or three quarters of liver tissue needs to be affected before a decrease in function occurs.[5]

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

Due to the public health importance of Toxoplasmosis, This study aimed to clarify the relationship of toxoplasmosis with liver diseases through knowledge of the incidence of *T. gondii* among Iraqi liver disease patients.

**Author for Correspondence: rawaasalim396@gmail.com*

MATERIALS AND METHODS

Samples

This study was included 204 samples of patients with liver disease attendees to the Gastroenterology and Liver Teaching hospital during the period from October 2018 to the end of January 2019, and their ages ranged from 13 to 75 years, five mL of venous blood were collected from all study groups (patients and control) using sterile syringes and then placed in gel tube and left for 30 minutes at the temperature (20–25), blood samples were centrifuged at 3000 rounds for five minutes then the serum collected and divided in eppendroff tubes and stored at -20℃ until they used.

Serological Tests

Toxo IgG/IgM Rapid Cassette Test

This assay is used to detect antibodies (IgG, IgM) in the patient sera as an initial diagnosis test, the kit is supplied by (CTK Biotech, USA).

ELISA for T. gondii IgG/IgM

Sera of this study were examined with ELISA test for detecting IgG and IgM antibodies; the kit was supplied by (Foresight, Germany).

Liver Function Tests

To determine the levels of liver enzymes (AST,ALT,ALP) in patients and control, the kit was supplied by (Siemens, Germany).

Statistical Analysis

The present study results were analyzed using SPSS (V.16). The result were represented as mean ± standard Deviation and significant value was calculated using one-way ANOVA and chi square to find the relationship between variables.

RESULTS AND DISCUSSION

Toxoplasmosis is an opportunistic protozoan disease, and the infection occurs all over the world. The clinical symptoms of infection variable from a symptomatic case to severe illness.[2,12,13] *T. gondii* affected different organs of the intermediate host like human, eyes, lymph nodes, liver, heart, central nervous system.[2,12,13] In the present study, the relationship between liver disease and toxoplasmosis in Iraqi patients has been identified by detecting IgG and IgM antibodies in serum using two diagnostic techniques: Immunochromatographic test (ICT) and enzyme linked immunosorbent assay (ELISA).

According to the Toxo IgG/IgM Rapid test, Table 1 shows the positive results positive of 50 patients with liver disease (24.5%) while the negative cases 154 (75.49%) with high significant differences (0.001).

The immunochromatographic test is mainly used to detect the presence of antibodies in sera of patients. Its quick and easy application characterizes it. Although this test is less expensive and effective for diagnosing parasitic diseases, is easy to perform by less experienced people, and is more rapid than conventional assay testing, it is less sensitive than other immunological assays. It can be useful in rapid initial screening of samples, and then positive results can follow other confirmatory tests such as other immunological and molecular techniques.[14]

Table 2 shows the results of ELISA test for IgG antibody. The positive cases were 49 positive cases in liver disease patients with a percentage of 24% while negative cases were 155 cases with a percentage (75.98%), without any significant differences.

ELISA is an exact serological tool for diagnosing toxoplasmosis according to antigen-antibody reaction can be evaluated by quantization of the color created by an ELISA reader, and the system is easy to perform, practical effectively adaptable for field use. ELISA is likewise appropriate for research centers required to examine huge number of samples,[15] detection of IgG antibodies allows to identify the infection in immunocompromised patients at risk of reactivation of latent infection by *T. gondii*, IgG titer persist in serum over 1 to 2 years, that provide information is to clinicians of the time of infection (new or had an infection in the past).[2,16]

IgG and IgM antibodies in liver diseases patients may be due to cellular and humoral immunity that is affected by chronic infection with the reactivation of latent infection with toxoplasmosis.[17,18] The current study results revealed a higher prevalence of IgG antibodies according to ELISA (24%). This is consistent with Tain *et al*.,[19] which observed a higher prevalence of IgG in patients with liver disease (19.7%). Also, it is consistent with El-Sayed *et al*.,[20,] which was conducted in Egypt on chronic liver patients. It shows the presence of IgG was 30%, and EL-Henawy *et al*.,[21] recorded that percentage of IgG were 32.8% in a patient with liver disease, Alvarado-Esquivel *et al*.,[22] showed seroprevalence IgG antibodies in liver disease patients (13.3%) this results may be due to small size of samples.

Patients with liver ailments are at risk of a wide range of bacterial, viral, and parasitic. Most types of CLD are joined by the downturn of both humoral and cellular immunity with checked powerlessness of attacking pathogens. During the parasitemia phase of *T. gondii* infection, the liver is

Table 1: Prevalence of Toxoplasmosis in liver disease patients according to the Toxo IgG/IgM rapid test

	Patients with liver disease		
Response	*No.*	*Percentage (%)*	*p-value*
Positive cases to IgG	50	24.5	0.001
Negative cases to IgM	154	75.49	
Total	204		

Note: The results were negative for IgM antibodies for all samples.

Table 2: Distribution of studied samples according to the presence of IgG with its significance comparisons.

	Patients with liver disease		
Response	*No.*	*Percentage %*	*p-value*
Positive cases	49	24	0.223
Negative cases	155	75.98	
Total	204		

one of the most significant organs included and influenced. The components of liver harm and the histological changes instigated by *T. gondii* infection could be because of a direct proliferative impact of the parasite on the tissues, prompting cell passing and tissue harm, or could be identified with the indirect impact of disease because of the excessive immunological reaction to the parasite. Some experimental studies have shown the presence of a tachyzoites phase and tissue cysts of the *T. gondii* parasite within liver cells and inside sinusoidal liver capillaries. When the parasite invades liver cells, this can lead to disturbances in metabolic activity and DNA damage.[20]

Table 3 shows 21 positive cases of IgM antibody in liver patients (10.29%) and negative cases 183 (89.70%) and significant differences (0.018).

IgM antibody in serum during infection with toxoplasmosis is very short in duration (few days to one week) and disappears within 3 to 5 months. This antibody refers to recent or primary infection by *T. gondii,*[18] the prevalence of IgM in the present study was (10.29%), it agrees with El-Nahas *et al*.,[23] which found that the percentage of IgM (13.6%) in late-stage and 12.8% in early-stage patients, In China, Tian *et al*.,[19] recorded the percentage of IgM which was (1.14). Alvarado-Esquivel *et al*.,[22] also showed that the prevalence of IgM antibodies in liver patients was 2.7%.

Table 4 shows 8 positive IgG and IgM antibodies (3.92%) and negative 196 (96.07%).

In this study, positive cases of IgG and IgM antibodies were (3.92%), Ustum *et al*.,[9] shows that the presence of both antibodies is an indicator of acute toxoplasmosis and may be a cause of liver damage and the onset of liver cirrhosis, according to the small percentage of antibodies present together.

Table 5 shows levels of liver enzymes (ALP, ALT, AST) in liver disease patients infected with toxoplasmosis, also liver disease patients without toxoplasmosis and control group.

Table 6 shows comparisons of liver enzymes between the studied groups.

Liver function tests (LFTs) are one of the most common blood tests, both for verifying suspected liver disease or controlling disease activity or simply as routine blood analysis,[24] El-Sayed *et al*.,[20] found in the study that the highest level of ALP enzyme was in chronic liver patients with toxoplasmosis 81.2 ± 0.7 IU/L while its level in chronic liver patients was 68.1 ± 1.8 IU/L and control group 74.1 ± 5.19 IU/L, Another study by El-Henawy *et al*.,[21] reported that the level of ALP in patients with hepatitis C with toxoplasmosis was 15.03 ± 5.10 IU/mL while its level in patients with hepatitis C was 12.80 ± 3.80 IU/mL. The results of these studies were low compared to the results of the current study. Elevated ALP in liver patients refers to the accumulation of bile salts or cholestasis and its production is in the cell membranes lining the bile ducts, while the reason for its rise in liver patients with toxoplasmosis is due to the presence of the parasite in the cells of the bile ducts, the site of production of ALP.[20,24] The present study observed an elevation in ALT levels in liver patients with toxoplasmosis, it was 57.72 ± 44.61 IU/L while its level in liver patients was 27.82 ± 46.55 IU/L and the control group was 14.01 ± 28.41 IU/L. El-Nahas *et al*.,(23) indicated that ALT level in early stage of hepatitis C patients (no cirrhosis) was 47.974 ± 32.400 IU/mL while its level in the late stage of the disease

Table 3: Distribution of studied samples according to the presence IgM with its signification comparisons.

	Patients with liver disease		
Response	*No.*	*Percentage (%)*	*p-value*
Positive cases	21	10.29	0.01
Negative cases	183	89.70	
Total	204		

Table 4: Distribution of studied samples according to the presence IgG and IgM with its significance comparisons.

	Patients with liver disease		
Response	*No.*	*Percentage (%)*	*p-value*
Positive cases	8	3.92	0.155
Negative cases	196	96.07	
Total	204		

Table 5: Liver enzymes levels that estimated in IU/L in all studied groups.

Liver enzymes	*Group*	*Mean*	*Standard Deviation*	*Standard Error*	*Minimum*	*Maximum*
ALP	Control	62.16	25.26	7.29	25	100
	Patients with liver disease	195.22	271.42	90.47	51	913
	Patients with liver disease and toxoplasmosis	162.5	76.95	22.21	68	309
ALT	Control	28.41	14.01	4.04	10	56
	Patients with liver disease	46.55	27.82	9.27	15	97
	Patients with liver disease and toxoplasmosis	57.72	44.61	13.45	18	152
AST	Control	22.5	8.47	2.44	10	35
	Patients with liver disease	53.88	43.11	14.37	26	143
	Patients with liver disease and toxoplasmosis	66.5	49.03	14.15	21	176

Table 6: Multiple comparisons of liver enzymes for potential pairs between study groups.

Liver enzyme	*Group 1*	*Group 2*	*Mean difference*	*Standard error*	*Sig.*
ALP	Control	Patients with liver disease	133.05	65.48	0.05
		Patients with liver disease and toxoplasmosis	100.33	60.62	0.108
	Patients with liver disease	Patients with liver disease and toxoplasmosis	32.72	65.48	0.621
ALT	Control	Patients with liver disease	18.13	13.76	0.198
		Patients with liver disease and toxoplasmosis	29.31	13.02	0.032
	Patients with liver disease	Patients with liver disease and toxoplasmosis	11.17	14.03	0.432
AST	Control	Patients with liver disease	31.38	16.52	0.067
		Patients with liver disease and toxoplasmosis	44	15.29	0.007
	Patients with liver disease	Patients with liver disease and toxoplasmosis	12.61	16.52	0.451

ALP- Alkaline phosphate
ALT- Alanine aminotransferase
AST- Aspartate aminotransferase

Table 7: Distribution of study samples depending on some epidemiological characteristics.

	Toxoplasmosis with liver disease		*Patient with liver disease*		
	N=78		*N=126*		
Variable	*No. Positive*	*Prevalence (%)*	*No. Negative*	*Prevalence (%)*	*p-value*
Gender					
Male	38	48.71	66	52.38	0.456
Female	40	51.28	60	47.61	
Age group (years)					
10–19	8	10.25	11	8.73	
20–29	20	25.64	26	20.63	
30–39	23	29.48	29	23.01	0.164
40–49	8	10.25	23	18.25	
50–59	11	14.10	14	11.11	
60–69	7	8.97	17	13.49	
70–79	1	1.28	6	4.76	
Job					
House maker	34	43.58	42	33.33	
Gainer					
Employee	13	16.66	32	25.39	0.001
Retired	24	30.76	29	23.01	
Student	1	1.28	8	6.34	
	6	7.69	15	11.90	
Type of disease					
HAV	6	7.69	4	3.17	
HBV	33	42.30	61	48.41	0.404
HCV	30	38.46	43	34.12	
CLD	9	11.53	18	14.28	

HAV- Hepatitis A virus
HBV- Hepatitis B virus
HCV- Hepatitis C virus
CLD- Chronic Liver disease

(disease progression to liver cirrhosis 41.803 ± 25.574 IU/mL, El-Henawy *et al*.[21] also found that the highest level of ALT in liver patients with toxoplasmosis was 51.65 ± 12 IU/mL, while in liver patients 55.20 ± 15 IU/mL, El-Sayed *et al*.,[20] reported that this enzyme level in liver disease patients with toxoplasmosis was 62.6 ± 11.28 IU/L and in liver patients 42 ± 8.42 IU/L, control group 23.2 ± 5.61 IU/L. These results are consistent with the results of the current study. The level of

AST in the present study was also higher in hepatitis patients with toxoplasmosis 49.03 ± 66.5 IU/L, whereas in the liver patients group 53.88 ± 43.11 IU/L and the control group had the lowest level of 8.47 ± 22.5 IU/L. El-Nahas *et al*.[23] showed the level of AST in patients with hepatitis C in the early stage 45.923 ± 30.462 IU/mL while Its level in the late stage of the disease was 57.370 ± 32.513 IU/mL. Also, the enzyme level was high in El-Henawy *et al*.,[21] study where 69.85 ± 21.30 IU/mL and in liver patients 64.81 ± 19.30 IU/mL, El-Sayed *et al*.,[20] study recorded the highest level in Liver patients with toxoplasmosis 78 ± 10.26 IU/L whereas in liver patients 53.49 ± 10.2 IU/L and control group 37.4 ± 1.15 IU/L, This consistent with the results of the current study. Elevated levels of ALT and AST are important signaling for liver cell injury, In cases of cellular damage these enzymes can leak into the blood and high levels may cause this due to damage in liver cells, liver infection with toxoplasmosis, may result in cholestasis, Swollen endothelial cells and Focal necrosis of liver cells.[20]

Some epidemiological characteristics were studied in this study, percentage of infection with toxoplasmosis was 51.282%, in patients with liver disease in female this agree with Tian *et al*.[19] which recorded the prevalence of toxoplasmosis in females are higher than in males (21.76, 19.16%), while El-Henawy *et al*.[21] found in his study in Egypt, a high infection of toxoplasmosis in males while in female was 38.9%. These results are inconsistent with the results of the current study, The customs and cultures of countries impact infection rates and its presence between gender.

In the term of age higher prevalence of infection in patients in age 30–39 (29.48%) this similar to El-Henawy *et al*.,[21] that shows the highest rate of infection was between 31–50 years (47.5%), while the study of Tian *et al*.[19] showed that the highest incidence of toxoplasmosis in the age group 41–50 years was (23.95%). The high rates of infection in young ages may be due to poor eating habits and eating contaminated food, which recorded this increase in liver disease in this age group of Iraqi patients. Older individuals had a highest susceptibility to exposure to toxoplasmosis risk than young people having smoking and drinking alcohol.[25] Also high prevalence of infection in this study is wth house marker (43.589%) with significant differences between the occupational factor and the injury. This may be due to the continued exposure to the risk factors of infection and transmission of the disease. Lack of sufficient information on how to deal with animals and breeding, especially cats roaming in the houses and direct contact with them, women not wearing gloves while cutting meat and lack of attention to hand hygiene.

According to the type of liver diseases, this study indicated that infection with toxoplasmosis in patient with hepatitis B (42.30%) and hepatitis C (38.46%) compared to other forms of liver disease, which agrees with study in Egypt, El-Sayed *et al*.,[20] which shows that high percentage of toxoplamosis infection in patients with HBV (33.3%), while in HCV (31.4%). El-Henawy *et al*.,[21] reported that the percentage of infection was 33.3% HCV and 11.1% HBV. These results might be because of the depletion of cellular immunity during chronic disease with HCV and HBV, resulting in reactivation of the latent *T. gondii* infection.[20]

REFERENCES

1. Dubey JP, Jones JL. *Toxoplasma gondii* infection in humans and animals in the United States. International Journal of Parasitology. 2008;38:1257-1278.
2. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. 2004; 363:1965-1976.
3. Dubey JP. Toxoplasmosis of Animals and Humans. 2nd ed. CRC Press, Taylor & Francis Group, USA. 2010. pp 336.
4. Dunay IR, Damatta RA, Fux B, Rachel P, Greco S, Colonna M, Sibley LD. Gr1+ inflammatory monocytes are required for mucosal resistance to the pathogen *Toxoplasma gondii*. Immunity. 2008;29(23):306-317.
5. Wedro B. Liver Disease Symptoms , Signs , Treatment , and Life Expectancy. 2019. www. medicinenet.com.
6. Weitberg AB, Alper JC, Diamond I, Fligiel Z. Acute granulomatous hepatitis in the course of acquired toxoplasmosis. The New England Journal Medicine. 1979;300:1093-1096.
7. Ortego TJ, Robey B, Morrison D, Chan C. Toxoplasmic chorioretinitis and hepatic granulomas. The American Journal Gastroenterology. 1990;85:1418-1420.
8. Tiwari I, Rolland CF, Popple AW. Cholestatic jaundice due to Toxoplasmic hepatitis. Postgraduat Medical Journal.1982;58: 299-300.
9. Ustun S, Aksoy U, Dagci H, Ersoz G. Incidence of toxoplasmosis in patients with cirrhosis. World Journal of Gastroenterology.2004; 10:452-454.
10. Wendum DN, Carbonell N, Svrcek M, Chazouilleres O, Flejou JF. Fatal disseminated toxoplasmosis in a *Toxoplasma* seropositive liver transplant recipient. Journal of Clinical Pathology. 2002;55:637.
11. Al-Jbouri AMS, Abood FM, Hindi NKK, Alkaim AF. Evaluation of antimicrobial activity of the aquatic extract against bacterial isolates from periodontitis in Babylon Province, Iraq ; Biochemical and Cellular Archives. 2018;18;1345-1350.
12. Kadhim MM, Kubba RM. Theoretical investigation on reaction pathway, biological activity, toxicity and NLO properties of diclofenac drug and its ionic carriers, Iraqi Journal of Science. 2020;61(5):936-951.
13. Al-Duhaidahawi DL, Jabir MS, Al-Amiery AA, Moneim AEA, Alkaim AF, Kadhum AAH. Novel antioxidants compounds derived from isatine Biochemical and Cellular Archives. 2018;18 (1);709-713.
14. El-Moamly AA. Immuno-chromatographic Techniques: Benefits for the Diagnosis of Parasitic Infections. Austin Chromatogr. 2014;1(4):1-8.
15. Sudan V, Jaiswal AK, Shanker D. Recent trends in the diagnosis of toxoplasmosis. Clinical Reviews and Opinions. 2013;5(2): 11-17.
16. Montoya JG. Laboratory diagnosis of *Toxoplasma gondii* infection and Toxoplasmosis. The Journal of Infectious Diseases. 2002;185(1): S73-82.
17. Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. Clinical Infectious Diseases. 1992;15:211-222.
18. Proter SB, Sande M. Toxoplasmosis of the central nervous system in the acquired immune deficiency syndrome. The New England Journal of Medicine. 1992;327:1643-1648.
19. Tian AL, Li GX, Elsheikha HM, Gardner DS, Zhang XY, Dong W, Yang XP, Luo YY, Li HL, Cong W, Zhu XQ. Seroepidemiolog

of *Toxoplasma gondii* infection in patients with liver disease in eastern China. Epidemiology and Infection. 2017;145(11): 2296-2302.

20. El-Sayed NM, Ramadan ME, Ramadan ME. *Toxoplasma gondii* Infection and Chronic Liver Disease :Evidence of an Association. Tropical Medicine and Infectious Disease. 2016;1(1):7 .
21. El-Henawy AA, Abdel-Razik A, Zakaria S, Elhammaday D, Saudy N, Azab MS. Is toxoplasmosis a potential risk factor for liver cirrhosis?. Asian Pacific Journal of Tropical Medicine. 2015;8(10):784-791.
22. Alvarado-Esquivel C, Torres-Berumen JL, Estrada-Martinez S, Liesenfeld O, Mercado-Suarez MF. *Toxoplasma gondii* infection and liver disease : A case- control study in a northern Mexican population. Parasite andVectors. 2011;4: 75.
23. El-Nahas HA, El-Tantawy NL, Farage RE, Alsalem AMA. *Toxoplasma gondii* infection among chronic hepatitis C patients: A case- control study. Asian pacific Journal Tropical Medicine. 2014;7(8):589-593.
24. Hall P, Cash J. What is the Real Function of the Liver Function, Tests? The Ulster Medical Journal. 2011;81(1):30-36.
25. Gajewski PD, Falkenstein M, Hengstler JG, Golka K. *Toxoplasma gondii* impairs memory in infected seniors. Brain Behavior and Immunity. 2014;36:193-199.