

Toxoplasmosis in thalassemic iraqi patients: serological and hematological study

Raghad N. Shihab¹, Israa Kasim Al-Aubaidi²

¹Mustansiriyah University, Iraqi Center for Cancer and Medical Genetic Research, Baghdad, Iraq

²Department of Biology, College of Education for Pure Science (Ibn-Al-Haitham), University of Baghdad, Baghdad, Iraq

ABSTRACT

Background. Toxoplasmosis is a zoonotic parasitic disease with high prevalence, it causes by an obligate intracellular parasite *Toxoplasma gondii*. Thalassemia is a blood disturbance has undergone through families in which the body makes an atypical form or sufficient amount of hemoglobin. The condition results in great numbers of red blood cells being damaged which cause to anemia. The purpose of this study was to identification the prevalence of *T. gondii* antibodies and hematological changes among thalassemic patients.

Methods. Samples were collected during March to June 2022 from Al-Karama Teaching Hospital in Baghdad, Iraq. After doctors diagnosis, necessary blood tests to detect thalassemia in a group of 165 thalassemic patients and 80 healthy controls. Serum specimens were investigated for Toxo IgM and IgG antibodies using immunochromatography test and chemiluminescent (CIMA) test, their age from 2-45 years.

Results. For immunochromatography test showed 44/165(26.67%) in thalassemic patients who have positive response for anti-Toxo IgG antibodies comparative with non-thalassemic control groups 33/80(41.25%) samples of have positive response for this test. The percentage infection with toxoplasmosis from thalassemia patients,60/165(36.36%) patients who have positive response for anti-Toxoplasma IgG antibodies as well as 25/80(31.25%) of non-thalassemic control group have positive response in chemiluminescent micro particle immunoassay(CMIA). However, result of CBC test showed that low significant levels in the group of thalassemic patients with toxoplasmosis with mean of Hb(8.286±0.128g/dl), MCV(62.027±2.146fL), MCH(23.111±0.327pg) and MCHC (26.888±0.385 g/dl) respectively in comparison with control group.

Keywords: toxoplasmosis, thalassemic, IgM, IgG, Hb and PCV

INTRODUCTION

The single-celled parasite *Toxoplasma gondii* is the cause of the toxoplasmosis infection. One of the most widespread parasite infections, it affects almost all warm-blooded creatures including humans and pets. During pregnancy, this parasite can be vertically passed to the fetus and the child may experience a wide range of clinical symptoms. It is an opportunistic pathogen in which the recurrence of the latent infection can cause death in congenitally infected fetuses, newborns, and immunocompromised patients [1-4]. *T. gondii* has three morphological forms, which are tachyzoites, bradyzoites and

sporozoites [5]. The intestinal phase of the parasite's life cycle occurs in the small intestine of cats, while the extra intestinal phase affects all intermediate hosts [6]. A series of genetic illnesses known as thalassemia cause a lower rate of - or -chain synthesis which partially or totally suppresses the rate of hemoglobin synthesis [7]. Thalassemia comes in two varieties: alpha and beta. Beta thalassemia major is an inherited disorder that may affect general health, gene mutations that result in low level and/or malfunctioning globin protein respectively, are the root causes of these disorders, one of these proteins might occasionally not exist at all Carriers of alpha

Corresponding author:

Raghad Shihab

E-mail: raghd.naji1102a@ihcoedu.uobaghdad.edu.iq

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or beta thalassemia trait exhibit minor symptoms depending on how severe the disease is, the human beta globin (HBB) gene, which is located on chromosome 11, regulates the form and functionality of hemoglobins, alpha thalassemia can lead to complications like hemolytic anemia or deadly hydrops fetalis [8-11].

Early infancy skeletal abnormalities one of the negative consequences of beta thalassemia major is hemolytic anemia, along with growth retardation. Children with this illness need frequent blood transfusions throughout their entire lives. Patients who need blood transfusions frequently suffer from iron overload, which can damage their liver or kidneys and cause other health problems.

Thalassemic patients who received blood transfusion are susceptible to acquiring toxoplasmosis, so blood transfusion is a source of a number of infections in some cases if the donor is infected with some serious diseases, especially parasitic infections [12].

The target of the present study was to determine the prevalence of *T. gondii* antibodies in thalassemic Iraqi patients and estimate hematological indicators among them.

MATERIALS AND METHODS

Subjects

This study was included cases were collected during March to June 2022 from Al-Karama Teaching Hospital in Baghdad, Iraq. After doctor's diagnosis, necessary blood tests to detect thalassemia. A group of 165 thalassemic patients and 80 healthy controls, their age range from 2-45 years.

Blood collection

Venous blood in the amount of 5 ml was taken from the sample. In order to separate the serum, three ml of the blood sample were immediately transferred to a gel tube and allowed to coagulate at room temperature (20–25°C) for 15 minutes, two ml of the blood sample were transferred to an EDTA-tube for haematological analysis.

Thalassemic diagnosis

Anti-coagulated blood samples were used to determine concentrations of red blood cells (RBC), Haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) by using CELL-DYN Ruby Haematology Analyzer system by the manufacturer Abbott.

T. gondii diagnosis

The procedures were carried out conferring to the kit manufacturer's protocol. *T.gondii* detected firstly by using Toxoplasma IgM/IgG antibody Immunochromatography rapid test kit (Qingdao High-top Biotech Company, China). According to the manufacturer's protocol followed by measuring the level of IgM/IgG via using chemiluminescent micro-particles immunoassay (CMIA) architect Toxo IgM/G kit (Abbott GmbH, Germany) depending to the manufacturer's instructions.

Ethical approval

This study was approved by Mustansiriyah University, Iraqi Center for Cancer and Medical Genetic Research, and Department of Biology, College of Education for Pure Science (Ibn-Al-Haitham), University of Baghdad (no 4004 in 2022).

Statistical Analysis

The impact of various factors on research parameters was determined using the Statistical Analysis System-SAS (2018) application [13]. To statistically compare between means, the Least Significant Difference (LSD) test (Analysis of Variation, ANOVA) was employed. In this study, the significant (P0.01, P0.05) probability was found using the Chi-square test.

RESULTS

Table 1 shown percentage of toxoplasmosis infection that 44/165 (26.67%) of the group of thalassemic patients has seropositive response for anti-Toxo IgG antibodies, also 33/80 (41.25%) of the group of non-thalassemic control has seropositive response for anti-Toxoplasma IgG antibodies with significant differences were observed in this test among the results ($P \leq 0.01$).

Moreover, Table 2 shown percentages of toxoplasmosis infections that 60/165 (36.36%) of the group of thalassemic patients who has positive response for anti-Toxoplasma IgG antibodies well 25/80 (31.25%) of the group of non-thalassemic has positive response for the same antibody. Significant differences observed in this test among the results ($P \leq 0.01$).

Furthermore, Table 3 revealed that the group of thalassemic patients with toxoplasmosis has the highest level of IgG antibody 41.475 ± 9.193 IU/mL according to CMIA followed by the group of non-thalassemic control positive which has the level of Toxoplasma IgG antibody 35.59 ± 8.336 IU/mL. However, all groups were seronegative response for anti-Toxoplasma IgM antibody.

TABLE 1. Immunochromatography Rapid test results of Toxoplasma IgM/IgG antibody in thalassemic and non-thalassemic groups

Group name	Total No. of samples for each group	IgG	IgM	IgG and IgM	P-value
Thalassemic patients	165	26.67% 44	00.0% 0	0.60% 1	0.0001 **
Non-thalassemic control	80	41.25% 33	1.25% 1	2.5% 2	0.0001 **
P-value	–	0.078 NS	0.802 NS	0.711 NS	–

** (P≤0.01) highly significant

TABLE 2. Percentage of Toxoplasma IgM/IgG antibodies in thalassemic and non-thalassemic groups

Group name	Total No. of samples for each group	IgG	IgM	IgG and IgM	P-value
Thalassemic patients	165	36.36% (60)	00.0% 0	0.6% 1	0.0001 **
Non-thalassemic control	80	41.2% 5% 33	1.2% 5% 1	2.5% 2	0.0001 **
P-value		0.0001 **	NS		

** (P≤0.01) highly significant

TABLE 3. Values of Architect Toxo – IgG and Toxo - IgM assay IU/mL in studied groups

Groups	No.	Mean ± SE IU/MI IgG	Mean ± SE IU/MI IgM
Thalassemic patients with toxoplasmosis	60	41.475±9.193	0.211± 0.098
Thalassemic patients	105	0.489±0.084	0.073±0.0022
Toxoplasmosis (control positive)	25	35.59±8.336	0.103±0.03
Healthy individuals (control negative)	55	0.5616±0.246	0.0422±0.004
LSD value	13.64 **	0.0662 *	
P-value	0.0056	0.0278	

* (P ≤0.05) significant, ** (P ≤0.01) highly significant

TABLE 4. Comparisons between thalassemic and non-thalassemic groups of hematological parameters values

Groups	Mean ± SE					
	RBC	Hb	PCV	MCV	MCH	MCHC
Thalassemic patients with toxoplasmosis	2.572±0.062	8.286±0.12	26.972±0.523	62.027±2.146	23.111±0.327	26.888±0.385
Thalassemic patients	2.586±0.026	8.134±0.53	26.674±0.348	66.5±0.843	23.348±0.194	26.542±0.236
Control positive (Toxoplasmosis)	4.106±0.062	13.216±0.109	39.566±0.334	90.014±0.589	29.703±0.118	33.206±0.099
Control negative (Healthy individuals)	5.134±0.064	13.024±0.098	39.52±0.255	93.846±0.514	29.73±0.109	33.65±0.108
LSD value	1.63 **	2.19 **	7.53 **	13.59 **	5.988 *	4.803b *
P-value	0.0056	0.0006	0.0022	0.0001	0.0289	0.0392

* (P ≤0.05) significant, ** (P ≤0.01) highly significant

The Table 4 clarified that highly significantly decreases of RBC, Hb, PCV and MCH (2.572±0.062, 8.286±0.12, 26.674±0.348 and 23.111±0.327) respectively in the group of thalassemic patients with toxoplasmosis, while the results showed decreases of RBC, Hb, PCV and MCH (2.586±0.026, 8.134±0.53,

26.674±0.348 and 23.348±0.194) respectively in the group of thalassemic patients were observed (P ≤0.01).

The percentages of distribution of the analyzed groups according to age categories are shown in tables 5 that clarified age range of 13-25 has a high

TABLE 5. Distribution of age characteristic in the studied group

Age range (Year)	Thalassemic patients with toxoplasmosis		Thalassemic patients		Toxoplasmosis patients (control positive)		Healthy individuals (control negative)		P-value
	No/60	%	No/105	%	No/25	%	No/55	%	
2-12	25	41.60	46	43.80	6	24.00	29	52.70	0.0001**
13-25	30	50.00	50	47.60	11	44.00	20	36.40	
26-38	4	6.70	8	7.70	4	16.00	4	7.30	
>39	1	1.70	1	0.90	4	16.00	2	3.60	
Mean ± SE	17.25± 8.40		17.25± 0.69		43.1 ± 2.90		32.76 ± 1.83		0.0001**

** (P<0.01) highly significant

percentage in all study groups 50.00%, 47.60% and 44.00% respectively in comparison with healthy control.

The results in Table 6 demonstrated the gender of the thalassemic patients. Among 35/60 of thalassemic patients with toxoplasmosis (58.30%) were males and 25/60 (41.70%) were females, while the percentage of thalassemic patients was 57.10 % male and 42.90% female categories. Furthermore, percentage of healthy individuals was 58.20% male and 41.80% female.

TABLE 6. Distribution of studied groups according to the gender characteristic

Groups	Male No. (%)	Female No. (%)	P-value
Thalassemic patients with toxoplasmosis	35 (58.30%)	25(41.70%)	0.025 *
Thalassemic patients	60(57.10%)	45(42.90%)	0.077
Toxoplasmosis patients (control positive)	14(56.00%)	11(44.00%)	0.152
Healthy individuals (control negative)	32 (58.20%)	23(41.80%)	0.025 *
P-value	0.0001 **	0.0097 **	---

* (P ≤0.05) significant, ** (P ≤0.01) highly significant

DISCUSSION

Toxoplasmosis has long been recognized as an opportunistic of illness in persons with impaired immune systems, blood transfusion infections are taken seriously, especially if they spread to people who receive blood transfusions frequently such as patients with thalassemia particularly in the acute stage, toxoplasmosis is thought to be a transfusion-transmissible infection among seropositive asymptomatic patients, it is normally asymptomatic in immunocompetent people, but it can cause substantial issues in immunocompromised people and can progress to a life-threatening infection [14-16].

In addition, the IgM/IgG antibodies immunochromatography rapid test is quick and simple utilized as a viable substitute screening tool for toxoplasmosis detection, results are acquired in 15 minutes without the need for equipment or experience and

it has a good specificity, positive or negative results in this test is determined by a visual colorimetric measurement made with the unaided eye. As a result of this qualitative or semi quantitative choice, false positive and false negative issues are unavoidable [17,18].

Additionally, the innovative anti-Toxoplasma IgG/IgM chemiluminescent microparticle immunoassay (CMIA), a fully automated method for evaluating the immunological status of the patients and ruling out either an acute or chronic infections, is used to identify Toxoplasma antibodies. It is regarded as a straight forward, inexpensive method with good sensitivity and specificity for toxoplasmosis screening [19]. Blood transfusion is a source of a number of infections in some cases if the donor is infected with some serious diseases, especially viruses and parasites, in which the blood is the main way of transport if it is taken from infected patients or contaminated from the environment or during the transfer. Acute parasitic infection in thalassemia patients can develop into a chronic stage that causes disorders in patients [20].

Toxoplasmosis has long been recognized as an opportunistic illness in persons with immunocompromised patients. It is a great importance, and that the disease does not show any clinical specific signs. It is also recognized as the third most common reason for AIDS patients to die, which makes it an important issue [21,22,23]. The seroprevalence of anti-Toxoplasma IgG revealed that out of 143 thalassemia patients screened, 28% (n=40) were seropositive for anti-T. gondii IgG antibodies. In stark contrast, only 5% (n=2) of healthy controls demonstrated prior exposure, as indicated by positive serological tests [24]. Abd El-Latif et al. [25] found that 45% (n=45) of patients had anti-Toxoplasma IgG antibodies without detectable IgM antibodies, while both anti-Toxoplasma IgM and IgG antibodies were present in 10% (n=10) of patients. IgM-only antibodies were discovered in 2% (n=2) of cases. The results of Kadhim et al. [26] reveal three groups based on IgG and IgM concentrations. For IgG, the highest concentration in males (164.63±25.0 IU/mL) was observed in the reactive group of beta-thalassemia patients with Toxoplasma, while the lowest concentration in fe-

males (98.276 ± 19.4 IU/mL) was also in the reactive group of beta-thalassemia patients with *Toxoplasma*. In beta-thalassemia patients without *Toxoplasma*, the highest concentration of IgG in males was 0.79 ± 0.17 IU/mL, and the lowest in females was 0.62 ± 0.15 IU/mL. Also the result of El-Tantawy et al. [27], who studied anti-*Toxoplasma* IgM and IgG in thalassemic children, 23.2% (49/211) had anti-*Toxoplasma* IgM and 53.6% (113/211) had anti-*Toxoplasma* IgG. While the prevalence of anti-*Toxoplasma* IgG in the positive control group was 51% (51/100), in the healthy control group, it was 5% (5/100) and for anti-*Toxoplasma* IgM, in the positive and control group, it was 51% (51/100), while in the healthy control group, it was 5% (5/100). This study agrees with the present study in the detection of anti-*Toxoplasma* IgG and lower percentage in the positive control group but disagrees in the detection of anti-*Toxoplasma* IgM.

According to the findings of Hanifehpour et al. [28], 55.31% of patients with thalassemia major and 37.02% of healthy individuals had anti-*Toxoplasma* IgG antibodies. Thalassemia patients may have greater anti-*Toxoplasma* IgG antibody titers than the control group because they are more likely to be at risk for *Toxoplasma* infection than healthy people because of frequent blood transfusions, thalassemic patients are to be compromised immune response against different type of infections, the reason for the differences in the results are not fully understood, but various factors such as environmental conditions, cultural habits, foods and safety level of the people against this parasite are the factors that can effect on the level of infection [29].

Karakas et al. [30], revealed that 7/36 (19.4%) of patients with thalassemic major patients and 5/36 (14%) of healthy control have seroprevalence rate of anti-Toxo IgG, however anti-Toxo IgM shown in 2/36 (5.5%) in thalassemic major patients.

The above results of table 4 may be attributed to the reduced beta globin chains in Hb molecules of thalassemic patients as a result, the structural alterations in Hb molecules patients which lead to RBCs characterized by excess boundless globin protein in cell membranes, this makes them subject to damage by phagocytic cells in the bone marrow, which could distinguish and damage abnormal cells leading to destruction of a great numbers of red blood cells through the process of erythropoiesis. Because thalassemia is a hereditary Hb synthesis defect that causes severe anemia in thalassemic patients, In the middle East and Mediterranean region, as well as more recently around the world [31]. Given the constant mobility of individuals to different parts of the world, particularly western countries, sickle cell-beta thalassemia (HbS/-thal) is a good example of a mixture of two common hereditary anemias [32].

Beta-thalassemia major patients may develop per oxidative tissue harm from repeated blood transfusions due to secondary iron excess [33]. Patients' hemoglobin levels dropped significantly, when compared to the values observed in controls, indicating that they needed blood transfusions, which was the main cause of iron excess [34]. MCV and MCH are highly linked to hypochromic microcytic anemia. Study of Fadhil et al [35]. Studied hematological parameters in thalassemic patients which noticed that Hb decreased in thalassemic patients (7.05 ± 1.43) g/dL in comparison with control groups (13.19 ± 0.95) g/dL. The present results of the study are in accordance with the study of Sari et al. [36] which showed reduced hematological parameters in the thalassemia patients.

The above of table 5 results attributed due to the patient who is up to 20 years is exposed to many complications, the most important of which is the increase iron overload. This result is agreed with previous study obtained by Al-Attar and Shekha [37] and Tawfeeq [38] Which explain that thalassemia major virtual can be diagnose completely within the early days or early months of age because the exhibitions of the disease may perform yet a complete switch from fetal to adult Hb synthesis occurs. The result of Al-Ghanimi et al. [39] appear a highly significant ($p \leq 0.05$) difference in the mean of All hematological parameters including RBC, HCT, MCV, MCH, MCHC and PLT except Hb was lower than the healthy group.

According to the gender there are no significant differences between gender because thalassemia it is a genetic disease that is transmitted from parents to offspring and to both genders equally. These results agreed with the previous study of Al-Attar and Shekha [37].

Saleh and Al-Numan [40] revealed that the prevalence rate of toxoplasmosis in 21/135 sample of thalassemic male patient's genders is (15.6%) while the rate in 21/115 sample of thalassemic percentage of infection among female's patients is (18.3%). However, the prevalence rate of toxoplasmosis in healthy male and female control were 13/30 (43.3%) and 9/20 (45%) respectively. The reason of the number increasing of infections in the gender group may be due to the frequency of transfusions.

CONCLUSION

In nations and places with low toxoplasmosis rates, the risk of transmission through blood transfusion is extremely low, and serologic testing for screening blood donors appears to be unnecessary. This study observe that *Toxoplasma* IgG levels were greater in all thalassemia patients than in the control group. However, the test of *Toxoplasma gondii*

was unaviable at Iraq's Baghdad transfusion centre. Given the high serologic infection rate of toxoplasmosis among thalassemia patients in this study, as well as the lack of effective medicines and a *T. gondii* vaccine, adequate techniques for minimizing the risk of infection are crucial. Blood screening

for *T. gondii* antibodies should be considered prior to transmission to patients, particularly in countries with a high toxoplasmosis incidence.

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