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Seroprevalence of *Toxoplasma gondii* among ocular infection patient in Shahid Dr. Aso Hospital in Sulaimani City, Iraq

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Abstract---*Toxoplasma gondii* is an intracellular protozoan parasite that causes ocular toxoplasmosis with most complications such as developing eye lesions and impaired vision. During the period from October 2021 to April 2022, a total of 174 blood samples were taken from ocular infected patients whom visited Shahid Dr. Aso Hospital in Sulaimani City, Iraq. These samples were tested serologically for detection of anti-*Toxoplasma gondii* antibodies (IgG and IgM) with both immunochromatography (rapid test) and ELISA. The serological test demonstrated that 63 IgG (36.2%) and 4 IgM (2.2%) were positive by rapid test immunochromatography. While, 80 IgG (45.9%) and 2 IgM (1.1%) were positive by ELISA. Most of the infections (58.75%) were between 50-69 years old. All patients (80) were examined for intraocular toxoplasmosis lesion, only 10 patients (12.5%) including 2 males (2.5%) and 8 females (10%) had ocular toxoplasmosis lesions. Most of the lesions were scars (old type) while, in some patients they were active lesions. The types of lesions, numbers per each eye, lesion diameter and comparison with the IgG and IgM titers were demonstrated. The present study indicates that ELISA diagnoses are highly sensitive to detect *T. gondii* than rapid test. In addition, the ocular toxoplasmosis lesions were more prevalent in the elderly and the number of the lesions increased with the age among ocular infected patients.

Keywords---*Toxoplasma gondii*, Ocular toxoplasmosis, Serology, Rapid test, ELISA, Color fundus photography, Chorioretinal scar.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa. It has been documented one of the most successful eukaryotic pathogen, that able to develop in a wide variety of vertebrate hosts [1]. Human and almost all homeothermic animals including birds and mammals serve as intermediate host, while the only definitive host for this parasite are house cat and certain other Felidae [2, 3].

Human infected by many ways such as: ingestion of tissue cysts in undercooked meat, ingestion of oocysts from contaminated water and food, blood transfusion, organ transplantation, accidental inoculation of tachyzoites and fetus infected through transmission of this parasite from infected mother via placenta [4]. It has been estimated that up to one-third of the world's human population harbors anti-T. gondii antibodies [5].

The primary method to diagnose toxoplasmosis is detection the positive titer of specific biomarker (T. gondii specific antibodies) in the patient serum including IgG and IgM [6]. Ocular toxoplasmosis (OT) is the most common and serious cause of retinitis and posterior uveitis, it can lead to blindness if the macula or optic discs are directly affected, or if vitreoretinal complications occur [7]. The clinical presentation of the interior side of the eye with the involvement of anterior segment are important tool for the diagnosis of ocular toxoplasmosis [8]. The common classic indications of ocular toxoplasmosis among adults is nidus of fluffy white necrotizing retinitis or retinochoroiditis close to a variably pigmented chorioretinal scar, while in children ocular lesion does not show any symptoms, or maybe lead to vision decline or pain in the infected eye [7]. This disease is more widespread in South and Central America and the Caribbean than in Europe and North America, and it's almost rare in China. Due to the existence of highly virulent parasite genotypes, ocular infection in South America is more severe than in other continents [9]. Iran as a neighbor of Iraq accounts for 10.1-19.4% or 5.4% among adults and children, respectively [10].

There are a few studies about OT in Iraq and they use different techniques to diagnose this disease [11-15], while the present study is the first study which conducted in Kurdistan Region of Iraq about OT. The aim of the present study is to determine the prevalence of toxoplasmosis among ocular infected patients whom visiting Shahid Dr. Aso Hospital (specialized for ocular consultancy) in Sulaimani City, Iraq based on positive detection of antibodies (IgG and IgM). Also, to reveal presence of ocular lesion among ocular toxoplasmosis patients and to compare the rapid test immunochromatograph to ELISA results to assess the sensitivity of immunochromatograph method in detection of T. gondii.

Method

Sample collection

The blood samples were collected from ocular infected patients (both genders) whom visited Shahid Dr. Aso Hospital in Sulaimani City, Kurdistan Region, Iraq, from October 2021 to the end of March 2022. Patient ages, sex, education level, were recorded. Venous blood (5 ml) was taken from each patient with disposable

plastic pyrogen free syringe. The blood transported into clot activator tube. The clot activator tubes were incubated at room temperature for 15-30 minutes to clot completely, then centrifuged at 2000 rpm for 10 minutes to obtain the serum.

Serological diagnosis

Diagnosis was established by laboratory examination and clinical examination. The laboratory examination was performed by examining of serum patients through using immunochromatography test (rapid test) and enzyme linked immunosorbent assay test (ELISA). Rapid test diagnosis was done by put a drop of patient's serum in to the cassette well and the result was recorded after 15-30 minutes according the protocol was provided to qualitative detection of *T. gondii* IgG/IgM antibody in the serum samples (Immunochromatography, Qinodao Hightop Biotech, China). For ELISA test, all sera samples were diluted 1:100 with IgG and IgM sample diluent which provided with the kit package according the manufacture protocol to presence and measure the level of those antibodies quantitatively. The value for IgG and IgM were expressed in IU/ml, and positive sample recorded when the titer was above 11 IU/ml (PISHTAZ TEB {PT}, Tehran, Iran).

Retinal examination

The clinical examination of the retina was conducted by preparing the patients, before examination, the pupil was dilated with dilating drop (Mydriacyl, Alcon eye drop, Swiss) to see clear view of retina. Once the data was collected, it was analyzed to rule out any results that were negative for anti-*T. gondii* IgG or IgM antibodies. Any patient who had positive results for one or both of them was asked to have their photo of the retina taken by a fundus camera (VISUCAM C Digital Camera ZEISS, Germany).

Statistical analysis

The results were tabulated and analyzed descriptively and statistically by using SPAA statistical program version 23.0 software (SPSS Inc. IBM, Chicago, Usa). T-test was used to comparison qualitative and quantitative data, P values <0.01 were considered statistically significant.

Ethical approval consent

All procedures in this study were conducted in accordance with the ethical committee of the College of Health & Medical Technology in Sulaimani Polytechnic University, which approved protocols (No. MLD 45 in 12/10/2021).

Result

A total of 174 ocular patients, 77 males (44.3%) and 97 females (55.7%), ranged between 1-75 years old were enrolled and screened for the presence of anti-*T. gondii* IgG and IgM antibodies. The demographic characteristics of investigated patients are shown in table 1. Among them, 80 cases (45.9%) were diagnosed with ocular toxoplasmosis. The serological test demonstrated that 63 IgG (36.2%) and

4 IgM (2.2%) were positive by immunochromatography (rapid test). While, 80 IgG (45.9%) and 2 IgM (1.1%) were positive by ELISA. There was a significant difference in serological detection of anti-*T. gondii* IgG between rapid test (RT) and ELISA (P value <0.01), while there was no significant difference (P value >0.01) between rapid test (RT) and ELISA in the detection of IgM. The comparison results of serological tests are shown in table 2.

All patients underwent detailed eye examinations and were searched for intraocular lesion and measurement of the lesions. Color retinography imaging was taken by photo fundus camera (VISUCAM C Digital Camera ZEISS, Germany). In the present investigation, from the 80 ocular toxoplasmosis patients, only 10 patients (12.5%), including 2 males (2.5%) and 8 females (10%) had ocular toxoplasmosis lesions. Most of the lesions were scars (old type) present as gray-blackish with hyperpigmented borders. While, in some patients they were active lesions which were delineated by whitish inflammatory focus arising at the border of a retinochoroidal scar also called stellate lesion after being infected again (Fig 1). Bilateral ocular toxoplasmosis was seen in 3 patients with old and active status. Types of lesions, numbers per each eye, lesion diameter and comparison with the IgG and IgM titers are shown in Table 3.

Table 1: General characteristics of the patients and distribution according to different classification criteria

Factor	Status	Frequency	Percent %
Sex	Male	77	44.3
	Female	97	55.7
Age (years)	1-13	18	10.3
	14-49	50	28.7
	50-69	86	49.4
	≥70	20	11.6
Educational level	Non*	3	1.7
	Illiterate	60	34.5
	Primary school	70	40.3
	High school	23	13.2
	University	18	10.3

* Infants who did not went to school.

Table 2: Seroprevalence of anti *T. gondii* antibodies (IgM and IgG) among ocular patients in Sulaimni City

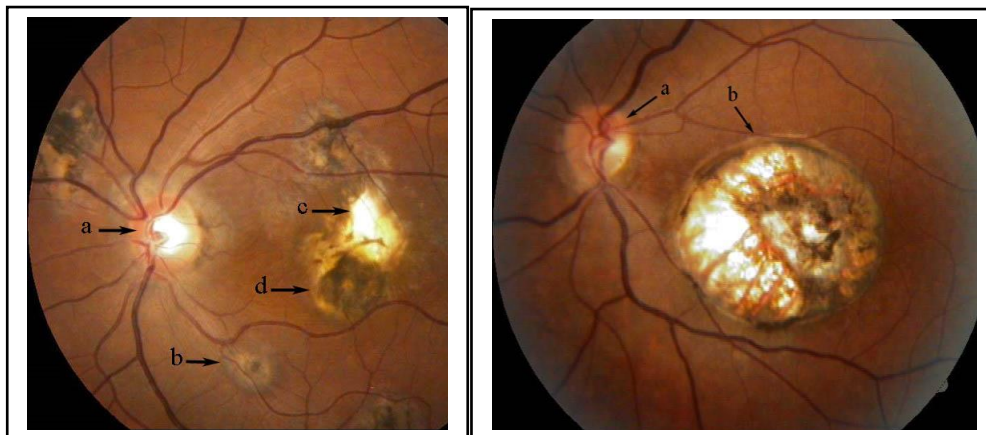
Serological Toxoplasma Test	IgG positive	IgM positive
Rapid Test	63 (36.2%)	4 (2.2%)
ELISA	80 (45.9%)	2 (1.1%)

The number and location of the lesions were studied and it appeared that, 4 cases out of 10 had only single retinochoroidal scar, while 6 cases had more than one scar. In addition, most of the retinochoroidal lesions were located adjacent to the optic nerve and macular area (Fig. 2) while, some lesions were peripherally located away from the center and outside of the macular area and only one case have complication and the lesion diffuse the retina completely.

Table 3: Information about ocular lesions among the patients in the present study

No. of ocular scar patient	Which eye infected?	No. of ocular scar in each eye	Ocular scar status	Position of ocular scar	Diameter of ocular scar (mm)	Age	Gender	IgG titer	IgM titer
Case-1	OD*	4	3 old, 1 active	peripheral	1.8	50-69	Female	273	4.2
Case-2	OS**	1	old	macula	1.5	14-49	Female	234	3.7
Case-3	OD	1	old	peripheral	1.03	14-49	Female	204	2.8
Case-4	OD	3	2 old, 1 active	Macula, peripheral	1.36-2.2	14-49	Female	124	6
Case-5	OD, OS	Full	old, active	Macula, peripheral	Spread totally	50-69	Female	172	3
Case-6	OS	3	old		0.44-1.85	50-69	Female	273	1.6
Case-7	OD, OS	8	7 old, 1 active	macula	2.1-5.04	14-49	Male	186	4.7
Case-8	OD, OS	2	old	Macula	1.1-3.75	50-69	Female	114	3
Case-9	OS	1	old	peripheral	0.39	50-69	Female	248	4.8
Case-10	OS	1	old	macula	0.71-1.1	14-49	Male	337	5.3

*OD (Oculus Dextrus) = right eye, **OS (Oculus Sinister) = left eye



Discussion

In the present study, the immunochromatography test (rapid test) was used as a diagnostic tool for the investigation of antibodies produced in response to *T. gondii* antigen [16]. While, enzyme linked immunosorbent assay test (ELISA) was used as a conformational idiagnostic tool because it has high specificity and sensitivity to detect antibodies against *T. gondii* [17-19]. In the present investigation, there was significant difference in the diagnosis of ocular toxoplasmosis by using ELISA and immunochromatography (rapid) methods and this finding was fit with the finding of Hassaneina *et al.* (2018) [16].

The prevalence of toxoplasmosis was evaluated in ocular patients, the result demonstrated that around half of patients were infected with toxoplasmosis, but most cases were chronic and only a couple of cases were acute or recurred with low IgM titer, based on serological test, It was found 43.1% were positive for IgG, while 1.1% for IgM. These findings could be due to the patient's visit ophthalmologist weeks or months after *T. gondii* infection and the titer of IgM

decrease when the patient came to the hospital, while IgG remain in high titer or remaining for a long time, this result is closed related to the finding of Mattos *et al.* (2011), Norouzi *et al.* (2017) and Lynch *et al.* (2002) whom they recorded high titers of IgG and low titers or not observed IgM by ELISA among their ocular toxoplasmosis patients [20-22].

In the present investigation, from 80 seropositive patients, only 10 (2 males and 8 females) (12.5%) presented ocular toxoplasmosis as an old or active lesion, this result closely resemble with the result of Samudio *et al.* (2015) in Brazil whom recorded 6 (5 males and 1 females) (8.9%) of ocular toxoplasmosis lesion among 80 ocular toxoplasmosis patients [23]. According to a new research that was done in 2020, the prevalence of retinochoroidal scars was higher in female patients than in males [24]. Also, the current study illustrates that females are more infected than males with ocular lesions. This may due to men were less likely than women to check their health at the local health unit. This statement was support by Passos *et al.* (2018) [25].

In this study, the macular region was the most commonly affected area by ocular toxoplasmosis lesions regarding to the total area of the retina, which is about 5% of the total area of the retina. Many studies agree on this concept, in which macular lesions are more frequent than other areas of the retina [7, 26]. The macula and the rest of the retina are very different in terms of anatomy and microcirculation. These characteristics make the macula more prone to the lesions, whether the infection was congenital or not [7].

The ocular toxoplasmosis lesions in the present study were more prevalent in the elderly and the number of the lesions increased with the age of the ocular infected patients, this finding is as the same of recorded by Bosch-Driessen *et al.* (2002) whom documented that ocular toxoplasmosis lesions were linked to the age of the patients [27]. Recurrence lesions were reported in two (20%) patients out of ten patients with ocular toxoplasmosis lesion, the ratio of recurrent toxoplasmosis lesions was different from a country to another country. Hosseini *et al.* (2018) reported 14.5% of recurrent ocular toxoplasmosis lesions among their patients [28]. The location of chorioretinal scars was mostly related to the center and macula of the eye that directly affecting the visual acuity and blindness [29]. It may due to a congenital infection. In general the central toxoplasmosis lesions affected 53% of the eyes, whereas peripheral were 40% [27]. The factor behind pattern and characteristics of the lesions produced by *T. gondii* is not clear [30]. However, it may be related to the host when the patient re-infected after consuming contaminated foods, bursting the old scar after altering in host immune status or the new strain can be blamed for the recurrence. People who did not get effective treatment have a greater chance of recurrence and producing new lesions beside the old ones [31, 32], and continuous multiplication of the parasite within the tissue cyst and rupturing as a result after a long time of infection and this leads to the formation of new lesions [33].

The data about OT in Iraq is very low, and they related to the prevalence of ocular toxoplasmosis among ocular infected patients in Ibn Al Haitham Teaching Hospital for Ophthalmologic Disease in Baghdad [11-15]. There is no any study about ocular toxoplasmosis in Kurdistan Region of Iraq. So, the present study

concerned to be the first study toward ocular toxoplasmosis among ocular infected patient in Kurdistan Region, Iraq.

Conclusion

It can be concluded that ELISA diagnoses are highly sensitive to detect *T. gondii* than rapid test. Also, *T. gondii* produce ocular lesion mostly near the macula and optic disc and ophthalmologists should consider diagnostic methods for identifying the infection when they visit the patients.

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