Real-Time PCR detection of Ocular Toxoplasmosis in Iraqi patients.

Issra K. Al-Aubaidi Ali N. Yaseen* Waheeda R. Ali

Department of Biology, College of Education for pure science (Ibn al-Haitham), University of Baghdad, Baghdad, Iraq.

*Department of Science , College of Basic Education, AL- Mustansiriya University , Baghdad, Iraq.

Abstract

Current study was done to investigate ocular toxoplasmosis that caused by *Toxoplasma gondii* parasite in blood samples of patients that reviewers to retina unit in Ibn- al Haitham teaching Eye hospital in Baghdad , using real-time PCR technique. Initially the disease was diagnosed by ELISA method using specific IgG and IgM antibodies for *T. gondii*. According to its positive results the studied groups were divided to ocular toxoplasmosis group that contain 42 patients, their proportion 84%, 14 patients in positive uveitis group in 31.1% percentage and control group recorded 35 positive case for infection with *T. gondii* in 41.7%percentage this group named as Asymptomatic toxoplasmosis group with significant differences ($p<0.01$) between the three groups. The highest mean of IgG recorded in ocular toxoplasmosis group 1.661±0.187 IU/ml and also for IgM 1.922±0.510 IU/ml. when Real-time PCR applied 14 positive results in ocular toxoplasmosis group were registered with 33.3 percentage against 7 positive results only for uveitis and Asymptomatic toxoplasmosis groups with rates 50% and 20% respectively without any significant differences , this test was successes in diagnosing of *T. gondii* parasite in different groups, although most infections were chronic with low rates in comparison with ELISA technique that recorded high rates of positive diagnosis of infection.

Key words: Ocular toxoplasmosis, Diagnosis, IgG, IgM, Real-Time PCR.

.

التحري عن داء مقوسات العين في المرضى العراقيين باستعمال تفاعل انزيم البلمرة المتسلسل في الوقت الحقيقي

اسراء قاسم العبيدي*علي نزار ياسين وحيدة رشيد علي
قسم علوم الحياة، كلية التربية للعلوم الصرفة (ابن الهيثم)، جامعة بغداد، بغداد، العراق
*قسم العلوم، كلية التربية الاساسية، الجامعة المستنصرية، بغداد، العراق

المستخلص

اجريت الدراسة الحالية للتحري عن داء مقوسات العين Ocular toxoplasmosis الناجم عن طفيلي *Toxoplasma gondii*في عينات دم المرضى المراجعين لوحدة الشبكية في مستشفى ابن الهيثم للعيون في بغداد وباستعمال طريقة تفاعل إنزيم البلمرة المتسلسل في الوقت الحقيقي Real-Time PCR. شخص المرض مبدئيا بطريقة الاليزاELISAوللأضداد النوعية IgG وIgM للمقوسة الكوندية،واستنادا لنتائجه الموجبة قسمتالمجاميع المدروسة الى مجموعة داء مقوسات العين عدد المرضى فيها 42 مريضا شكلوا نسبة 84% و14 مريضا من مجموعة التهابات العنبية Uveitis شكلوا نسبة 31.1% ومجموعة السيطرة التي سجلت 35 حالة موجبة للمقوسة الكوندية شكلت نسبة 41.7% وسميت بمجموعة داء المقوسات عديمة الاعراض Asymptomatic Toxoplasmosis وبفارق معنوي عند احتمالية ($p<0.01$)بين المجاميع الثلاثة ، بلغ اعلى معدل الضد IgGلدى مجموعة داء مقوسات العين 1.661 ± 0.187 وحدة عالمية /مل وكذلك الحال للضد IgMالذي بلغ معدله فيها1.922±0.510 وحدة عالمية /مل. وعند تطبيق تفاعل إنزيم البلمرة المتسلسل في الوقت الحقيقيسجلت 14نتيجة موجبة لمجموعة مرضى داء مقوسات العين وبنسبة 33.3 % مقابل 7 نتائج موجبة فقط لمجموعتي التهابات العنبية وداء المقوسات عديمة الاعراض شكلوا نسبتي 50 % و 20% على التوالي ، مع عدم تسجيل فروقات معنوية بينالمجاميع ($p<0.107$) ،نجح هذا الاختبار من تشخيص تواجد طفيلي المقوسة الكوندية لدى مختلف المجاميع بالرغم من اغلب الاصابات كانت مزمنة chronic وبنسب قليلة مقارنة مع اختبار الاليزا الذي سجل ايجابية عالية في تشخيص الاصابة.

الكلمات المفتاحية: داء مقوسات العين، التشخيص ، IgG،IgM،تفاعل انزيم البلمرة المتسلسل في الوقت الحقيقي

Introduction

Toxoplasmosis a disease resulting from infection by the protozoan *Toxoplasma gondii,* which infects, both humans and warm-blooded animals as a zoonotic pathogen a worldwide distribution (1,2). Approximately one-third of all humanity has been exposed to this parasite and this disease is listed as the third biggest cause of life threating food borne disease (3). *T. gondii* has a complex life cycle that includes an asexual and sexual cycles , the asexual cycle occurs in a wide range of intermediate hosts and the sexual cycleoccurs exclusively in feline hosts, which shed infectious oocysts in their feces(4).There are three major ways of transmission 1: consuming food or water containing oocyst 2: eating undercooked meats containing tissue cysts and 3: transmission via placenta (1). In humans, *Toxoplasma* infections are widespread and can lead to severe disease in individuals with an immature or suppressed immune system, (2,4).

Ocular toxoplasmosis (O.T.) or, more precisely, toxoplasmic retinochoroiditis is the most frequent cause of infectious blindness and visual morbidity amongst young adults in developed countries and it's associated with both congenital and acquired infections (5,6). O.T. is characterized by necrotizing retinopathy, which is triggered by the activation of dormant organisms within the retina (5). Active lesions that are accompanied by a severe vitreous inflammatory reaction will have the classic "head light in the fog" appearance. It's the main cause of posterior uveitis and can cause serious sequelae including complete loss of vision (6,7).

Clinical ophthalmological findings together with positive anti – *T. gondii* serology provide sufficient information on which to base a diagnosis (8).Cell culture of intraocular fluids is particularly intensitive when any a small amount of materials is available but it may take days to weeks to obtain a result. PCR detects the DNAs of microorganisms and is a rapid method which has been used to detect *T. gondii* in different biological samples (9).The aim of the present work was to detect toxoplasmic DNA on peripheral blood of patients with ocular toxoplasmosis.

Materials and Methods

The current study was conductedon95 patients whose underwent to the retina unit at Ibn Al- Haitham hospital of eyes in Baghdad, Iraq, from March to September 2013. All patients were clinically diagnosed by physician using slit-lamp bio microscopy device and binocular ophthalmoscopy with the expansion of the iris maximum

mydriasis. Healthy eighty four subjects were considering as control group. Age ranged from 10 to 70 in patients and control groups. Five ml of venous blood were drawn from patients and healthy individuals , each sample was divided in two tubes , the first one contain 3 ml of blood for immunological diagnosis (ELISA) and the second one contain 2 ml blood which placed in sterilized EDTA tube for molecular diagnosis. The sera of all cases were tested for the presence of specific IgM and IgG anti-*Toxoplasma* antibodies via ELISA kits (Bio check diagnostics company, USA)according to the manufacturer's instructions.

Isolation of genomic DNA from whole blood / DNA was extracted from the whole blood samples of the study groups using a commercial purification system (*AccuPrep*® Genomic DNA Extraction Kit, Bioneer , Korea) following the manufacture's instruction for DNA purification from blood. For Real-Time PCR assay, commercial quality quantitative detection of DNA parasite *Toxoplasma gondii* (AccuPower® TG Real - Time PCR Kit) from the production of Korean company Bioneer was used (Table 1).

Table (1): Specialcomponents of Real-Time PCR kit.

Component	Quantity
TG PCR Premix (primers,probes , DNA polymerase, dNTPS, salts)	8- well strip x 12 ea
TG Positive Control (PC) DNA	15 µl / tube x 2 strips (Natural)
Internal Positive Control (IPC) DNA	15 µl / tube x 2 strips (Yellow)
DEPC DW (No Template Control, NTC)	15 µl / tube x 2 strips (Purple)
DEPC DW	1800 µl / tube x 4 ea
SL buffer	1800 µl / tube x 4 ea
Optical Sealing film	1 ea

The TG PCR Premix mix was prepared for each sample according to company instruction as following table (2).

Table (2): Components of TG PCR Premix mix.

Component	Volume
TG PCR Premix	Number of tubes
Internal Positive Control (IPC) DNA	1µl
DEPC DW	44µl
Genomic DNA template	5µl
Total volume	**50µl**

Then tubes placed Exispin vortex centrifuge at 2500 rpm for 5 minutes, after that transferred into Exicycler™ 96 Real-Time PCR and applied the following thermo cycler conditions as the following table (3).

Table (3): Thermo cycler conditions.

Step	Function	Temperature	Time (hh:mm:ss)	Repeat
1	INCUBATE	95.00	0:5:0	0
2	INCUBATE	95.00	0:0:5	0
3	INCUBATE	55.00	0:0:5	0
4	SCAN			0
5	GOTO	Step2		45
6	INCUBATE	25.00	0:1:0	0
7	End			

Statistical analyses were computer assisted using the Statistical Package Statistical Package for Science SPSS version 2010, and included the following statistical tests: Descriptive statistical tables: Mean, Standard Error, Standard Deviation, 95%Confidence Interval of the Mean and Contingency Coefficient (C.C.) by $P>0.05$ and $P<0.01$ was considered statistically significant.

Results

In the present study, 50 samples of sera from patients with ocular toxoplasmosis, 45 samples from uveitis and 84 samples was consider as a control group , were tested by ELISA specific IgM and IgG antibodies for *T.gondii* and by Real-Time PCR technique. As shown in table (1), positive and negative results were recorded, positive IgM and IgG in O.T. group was 42(84%) , while in uveitis 14(31.1%) and 35(41.7%) for control .Negative ELISA IgM and IgG, distributed as 8 (16%) for O.T. group, 31(68.9%) for uveitis and 49(58.3%) for control group. According to ELISA test the groups was renamed as shown in tables two and three. The cut – off value of positive

IgM and IgG was 1.00 IU/ml., this point used to differentiate positive results from negative results. Table (2) showed results of the level of IgG antibody in all studied groups. Higher results was recorded in O.T. group 1.661 ± 0.187 I.U./ml., followed by positive uveitisgroup1.596 ± 0.157 I.U./ml. and Asymptomatic toxoplasmosis group withvalue1.501 ± 0.257I.U./ml. while other groups presented low results of this antibody (Table 2), this table explained also the lower and upper limit of IgG level in all groups.

Levels of IgM antibody was detected in this study (Table 3). Patient of Ocular toxoplasmosis group recorded higher level of IgM in a value 1.922 ± 0.510 I.U./ml. followed by positive Uveitis group1.867 ± 0.061 I.U./ml., and finally Asymptomatic toxoplasmosis group which recorded 1.744 ± 0.070 I.U./ml., these patients were had acute toxoplasmosis,5.While negative results for all groups was represented in this table also. Table (3) shown lower and upper limit of IgM antibody in all studied groups. Some pathological effects of *T. gondii* infection in eyes of O.T. patients was documented in images (Figure 2,3).

Besides the serological diagnosis of O.T. Real-Time PCR technique was used to confirm the infection with *T. gondii* by detection of *T. gondii* DNA in the blood samples of patients and control, the study revealed that out of 42 patients with O.T. only 14(33.3%) showed positive results by R.T.-PCR technique while 7(50%) and 7(20%) for positive uveitis and Asymptomatic toxoplasmosis respectively (Table 4, Fig.3). Statistically, there was no significant differences between them ($p<0.05$).

Discussion

Ocular Toxoplasmosis is a major cause of posterior uveitis worldwide but its incidence and prevalence are difficult to evaluate precisely (6).The diagnosis is usually based on ophthalmological examination showing unilateral, whitish, fuzzy-edged, round, and focal lesions surrounded by retinal edema (7). Laboratory diagnosis is based on the detected of antibody profiles in ocular fluid or in serum samples but it has many limitation(8,9,10).The results of the present study showed the presence of IgM and IgG antibodies in the sera of all studied groups with some variations (Table 1,2,3) but IgG antibody was detected in all groups with high percentages especially in patients with O.T. 83.33% and 85.71% in positive uveitis and Asymptomatic toxoplasmosis groups respectively, this finding referred that immunoglobulin G was the major class involved in the humoral immune response to against the *T. gondii* parasite, this is similar to the findings ofAl-Azawi *et al.*(11) and Al-Hakeem(12) which found the presence of IgG antibody was higher.

The detection of specific IgM antibodies has been the most frequently used serological marker for diagnosing recent toxoplasmosis (13). In current study the presence of specific IgM antibodies was low in all groups. Only 7 cases have positive results in O.T. group, 2 cases in positive uveitis group and 5 cases in control group. Other studies recorded this low rate also, Ronday *et al.*(14) detected only one case of IgM, Ongkosuwito *et al.*(15) diagnosed a rate 8% of positive IgM from 24 patients of ocular toxoplasmosis, Al-Azawi*et al.*(11) recorded a percentage 4.8% of positive IgM from 42patients of Toxoplasmicchorio retinitis, and this was similar to the results of the present study.

The real-time PCR, has grown considerably over recent years, the technique had proven to be useful for the early and accurate diagnosis of toxoplasmosis and for guiding pre-emptive therapy in patients at high risk of developing invasive disease(16). Peripheral blood samples originated from patients that gave positive results for specific IgG and IgM toxoplasmosis were analyzed by rael-time PCR. The results showed few patients were positive for *Toxoplasma* parasitemia on blood samples (Table 4), only 33.3%, 50% and 28% percentages for O.T., uveitis and asymptomatic toxoplasmosis groups respectively. These results were in agreement with Cardona *e t al*. (17) that found 18.18% (4/22) of cases with cerebral toxoplasmosis were positive by R.T. PCR and concluded that, real-time PCR on peripheral blood samples was not useful for diagnosis of cerebral toxoplasmosis. In the study of Bou*et al.*(18) they found a positive PCR result was obtained with the

blood from 8 of 15 (53.3%) patients with the diagnosis of ocular toxoplasmosis . Similar finding have been reported by Dupouy – Camet*et al*.(19). Moreover other studies (20,21,22,23) showed sensitivity values of tests with blood , 10 to 35% . Fekkar *et al*.(24) obtained only 8% of *T. gondii* detection on blood for diagnosis of ocular toxoplasmosis by PCR , and considered that PCR detection of *T.gondii* DNA in blood samples cannot be a sufficient tool for the diagnosis of ocular toxoplasmosis , and these similar to the results of this study. The low percentage of diagnosis may be explained that ocular toxoplasmosis is considered a local event in eye (25) and there is no in significant systematic parasitemia, instead most probably there exists a local reactivation (26), and most of patients were during the chronic phase of infection.
In the present study *T.gondii* DNA in the blood of some patients of uveitis and asymptomatic toxoplasmosis groups were detected (Table 4). In order to explain this result, it is possible that a small number of parasites might be released from tissues into the blood at subclinical level, and their presence can be detected by PCR as low-level immunosuppressive states (18).

References

1- Robert- Gangenux, F. and Dardé, M. L. (2012). Epidemiology of and Diagnostic Strategies for Toxoplasmosis. Clin. Microbiol. Rev., 25(2):264-296.

2- Rosso, F.; Les, J.T.; Agudelo, A.; Villalobos, C.; Chaves, J.A.; Tunubala,G.A.; Messa, A.; Remington, J.S. and Montoya, J.G. (2008). Prevalence of infection with *Toxoplasma gondii* among pregnant women in Cali, Colombia, South America. Am. J. Trop. Med. Hyg.,78(3):504–508.

3- Herrmann, D.C.; Pantchev, N.;GlobokarVrhovec, M.; Barutzki, D.; Wilking, H.; Frhlich, A.; Lüder, C.G.K.; Conraths, F.J. and Schares, G.(2010). Atypical*Toxoplasma gondii* genotypes identified in oocysts shed by cats in Germany. Int. J. Parasitol., 40: 285–292.

4- Sullivan Jr,W.J. and Jeffers,V. (2012). Mechanisms of *Toxoplasma gondii*persistence and latency. F.E.M.S. Microbiol. Rev. , 36: 717–733.

5- Holland, G. N. (2003). Ocular toxoplasmosis: a global reassessment. Part I: epidemiology and course of disease. Am. J. Ophthalmol., 136: 973-988.

6- Peterson, E.; Kiljsh, A. and Stanford, M. (2012). Epidemiology of ocular toxoplasmosis. Ocul. Immunol. Inflamm., 20(2): 68-75.

7- Butler, N. J.; Furtado, J. M.; Winthrop, K. L. and Smith, J. R. (2013). Ocular toxoplasmosis II: clinical features, pathology and management. Clin. Exp. Ophthalmol., 41(1): 95-108.

8- Weiss, L. M. and Kim, K. (2007). *Toxoplasma gondii* the model apicomplexan: perspectives and methods, Elsevier, Amsterdam: 777 pp.

9-Talabani, H.; Asseraf, M.; Year, H.; Delair, E.; Ancelle, T.; Thulliez, P.; Bre`zin, A. P. and Dupouy-Camet, J. (2009). Contributions of immunoblotting, real-time PCR, and the Goldmann-Witmer coefficient to diagnosis of atypical toxoplasmic retinochoroiditis. J. Clin. Microbiol., 47: 2131-2135.

10-Garweg, J. G.; De Groot-Mijnes, J. D. F. and Montoya, J. G. (2011). Diagnostic approach to ocular toxoplasmosis. Ocul. Immunol. Inflamm., 19(4): 255-261.

11-Al-Azawi, A. K. A.; Al-Rawe, I. H. A. and Al-Bayati, R. Y. J. (2013). Sero-prevalance of toxoplasmic chorioretinitis in Baghdad province. Int. J. Sci. Nat., 4(1): 68-71.

12-Al-Hakeem, A.F.S. (2015).Prevalence of Ocular Toxoplasmosis and Evaluation of Some Immune Status Aspects in Samples of Iraqi Patients. Msc. Thesis, Coll.Edu. Pure Sci.(Ibn al- Haitham),Univ. Baghdad:146pp. (In Arabic).

13- Balsari, A.; Poli, G.; Molina, V.; Dovis, M.; Petruzzelli, E.; Boniolo, A. and Rolleri, E. (1980). ELISA for *Toxoplasma* antibody detection: a comparison with other serodiagnostic tests. J. Clin. Pathol., 33(7): 640-643.

14-Ronday, M.J.; Ongkosuwito, J.V.; Rothova, A. & Kijlstra, A. (1999). Intraocular anti- *Toxoplasma gondii* IgA antibody production in patients with ocular toxoplasmosis. Am. J. Ophthalmol., 127(3):294-300.

15-Ongkosuwito, J.V.; Bosch-Driessen, E.H.; Kijlstra, A. & Rothova, A. (1999).
Serologic evaluation of patients with primary and recurrent ocular toxoplasmosis for evidence of recent infection. Am. J. Ophthalmol.,128:407-412.

16- Martino,R.; Bretagne, S.;Einsele, H. ; Maertens, J.; Ullmann, A. J. & Parody, R.(2005). Early detection of *Toxoplasma* infection by molecular monitoring of *Toxoplasma gondii* in peripheral blood samples after allogeneic stem cell transplantation. Clin. Infect.Dis., 40: 67–78.

17- Cardona,N.; Basto,N.; Parra,B.; Zea,A.F.; Pardo,C.A.; Bonelo,A. &G´omez-Marin, J.E.(2011).Detection of *Toxoplasma* DNA in the Peripheral Blood of HIV-Positive Patients with Neuro-opportunistic Infections by a Real-Time PCR Assay. J. Neuroparasitology. 2: 1-6.

18-Bou,G. ; Figueroa,M.S. ; Marti´-Belda, P. ;Navas,E.and Guerrero, A.(1999).Value of PCR for Detection of *Toxoplasma gondii*in AqueouHumor and Blood Samples from Immunocompetent Patientswith Ocular Toxoplasmosis. J. Clin. Microbiol.,37: 3465-68.

19- Dupouy-Camet, J. ; de Souza, S. L.;Maslo, C.;Paugam, A. ;Saimot, A.;Benarous, R. ;Tourte-Schaefer, C.&Derouin, F.(1993). Detection of *Toxoplasma gondii* in venous blood from AIDS patients by polymerase chain reaction. J. Clin. Microbiol.,31:1866-1869.

20-Dupon, M. ;Cazenave, J.;Pellegrin, J. L. ;Ragnaud, J. M. ;Cheyrou, A. ; Fischer, I.; Leng, B. & Lacut, J. Y.(1995). Detection of *Toxoplasma gondii* by PCR and tissue culture in cerebrospinal fluid and blood of human immunodeficiency virus-seropositive patients. J. Clin. Microbiol., 33:2421-2426.

21-Filice, G. A. ;Hitt, J. A. ; Mitchell, C. D. ;Blackstad, M. & Sorensen, S. W.(1993). Diagnosis of toxoplasma parasitemia in patients with AIDS by gene detection after amplification with polymerase chain reaction. J.Clin.Microbiol.,31:2327-2331.

22- Franzen, C. ;Altfeld, M.;Hegener, P.; Hartmann, P.; Arendt, G. ;Jablonowski, H.;Rockstroh, J.; Diehl, V.; Salzberger, B. & Fatkenheuer, G.(1997). Limited value of PCR for detection of *Toxoplasma gondii* in blood from human immunodeficiency virus-infected patients. J. Clin.Microbiol. ,35:2639-2641.

23-Guy, E. C. &Joynson, D. H.(1995). Potential of the polymerase chain reaction in the diagnosis of active *Toxoplasma* infection by detection of parasite in blood. J. Infect. Dis.,172:319-322.

24-Fekkar, A.; Bodaghi, B.; Touafek, F.; Le Hoang, P.; Mazier, D. & Paris, L.(2008). Comparison of immunoblotting, calculation of the Goldmann-Witmer coefficient, and real-time PCR using aqueous humor samples for diagnosis of ocular toxoplasmosis. J. Clin. Microbiol., 46: 1965-1967.

25-Contini, C.; Seraceni, S. ;Cultrera, R.; Incorvaia, C.; Sebastiani, A. & Picot, S. (2005). Evaluation of a Real-time PCR-based assay using the light cycler system for detection of *Toxoplasma gondii* bradyzoite genes in blood specimens from patients with toxoplasmic retinochoroiditis. Int. J. Parasitol., 35:275-283.

26-Silveira, C.;Vallochi, A. L. ; Rodrigues da Silva, U.; Muccioli, C.; Holland, G. N. ; Nussenblatt, R. B. ; Belfort, R. & Rizzo, L. V. (2011). *Toxoplasma gondii* in the peripheral blood of patients with acute and chronic toxoplasmosis. Br. J. Ophthalmol., 95:396-400.

Table: (1) The ELISA assay and percentages for all the study groups.

Groups	No. and	ELISA Result	Total	

	Percent	Positive	Negative		C.C.& P-value
O.T.	No.	42	8	50	
	%	84	16	100	
Uveitis	No.	14	31	45	
	%	31.1	68.9	100	C.C. = 0.389
Control	No.	35	49	84	P=0.000
	%	41.7	58.3	100	H.S.
Total	No.	91	88	179	
	%	50.8	49.2	100	

H.S.: Highly Significant at P< 0.01

Table (2): Levels of IgG antibody by IU/ml for all study groups.

Groups	The Total No. of Samples	No. of Positive Samples	Mean	Standard Deviation	Standard Error	%95 Confidence Interval for Mean		Lower Value	Upper Value
						Lower Bound	Upper Bound		
O.T.	42	35	1.661	0.187	0.032	1.597	1.7	1.319	2.143
Uveitis +	14	12	1.596	0.157	0.045	1.496	1.7	1.344	1.831
Uveitis -	31	-	0.389	0.19	0.034	0.319	0.5	0.123	0.702
Asymptomatic Toxoplasmosis	35	30	1.501	0.257	0.047	1.405	1.6	1.184	1.897
Control	49	-	0.35	0.167	0.024	0.302	0.4	0.122	0.712

Table (3): Levels of IgM antibody by IU/ml for all study groups.

Groups	The Total No. of Samples	No. of Positive Samples	Mean	Std. Dev.	Std. Error	%95 Confidence Interval for Mean		Lower Value	Upper Value
						Lower Bound	Upper Bound		
O.T.	42	7	1.922	0.51	0.193	1.45	2.4	1.472	2.658
Uveitis +	14	2	1.867	0.061	0.043	1.321	2.4	1.824	1.91
Uveit is -	31	-	0.632	0.166	0.03	0.571	0.5	0.306	0.768
Asymptomatic Toxoplasmosis	35	5	1.744	0.07	0.031	1.657	1.8	1.679	1.858
Control	49	-	0.618	0.236	0.034	0.55	0.7	0.238	0.762

Table (4): Results of Real - time PCR test and percentagesforpositiveELISAstudy groups.

Groups	No. and Percent	Real-timePCRResult		Total	C.C.& P-value
		Positive	Negative		
O.T.	No.	14	28	42	C.C. = 0.216 P=0.107 NS
	%	33.3	66.7	100	
Uveitis +	No.	7	7	14	
	%	50	50	100	
Asymptomatic Toxoplasmosis	No.	7	28	35	
	%	20	80	100	
Total	No.	28	63	91	
	%	30.8	69.2	100	

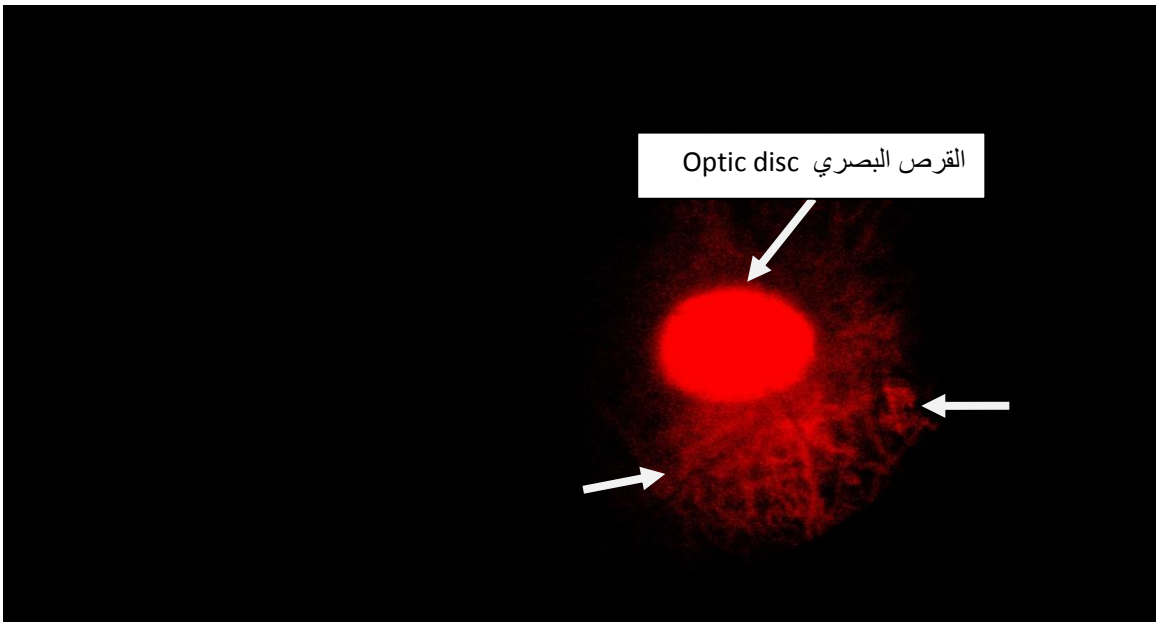
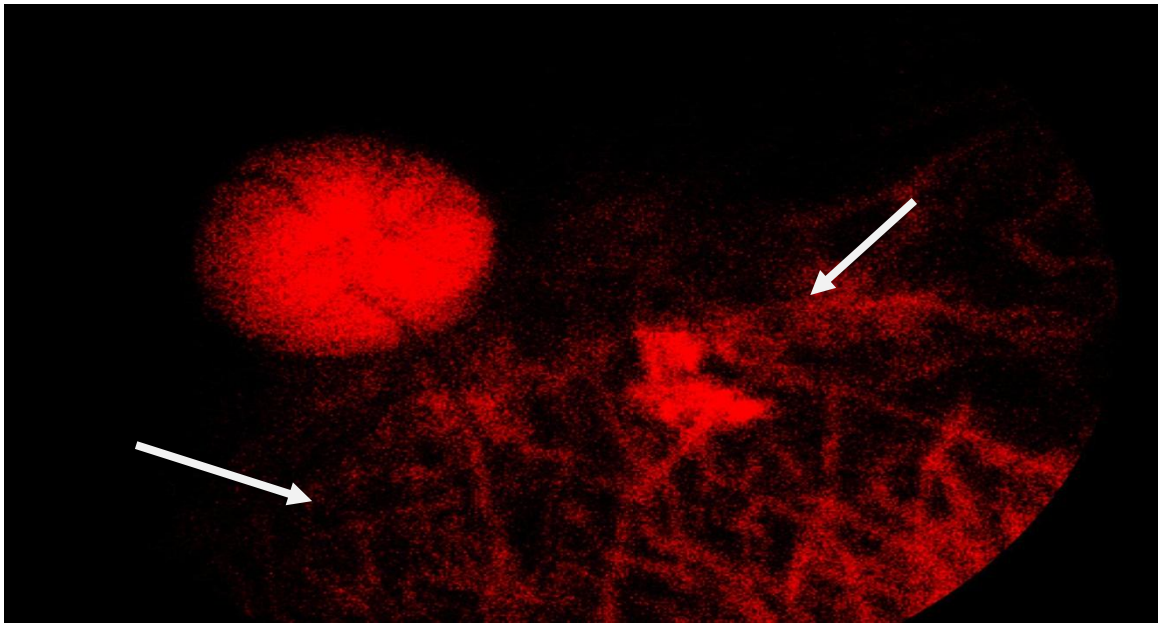
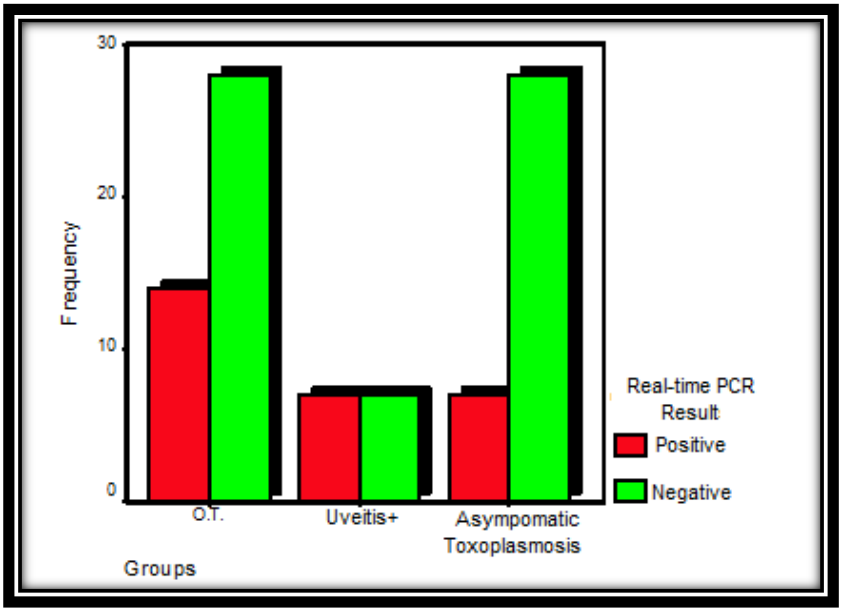


Figure (1): Scar diffused in the retinaas a result of OcularToxoplasmosis infection.



Figure(2): The spread ofmanyscares inthe retinadue tochronicOcular Toxoplasmosisinfection.



Figure(3): graphictapes for the distribution of frequencies in accordance with the percentage Real-time PCR test for positive ELISA study groups.

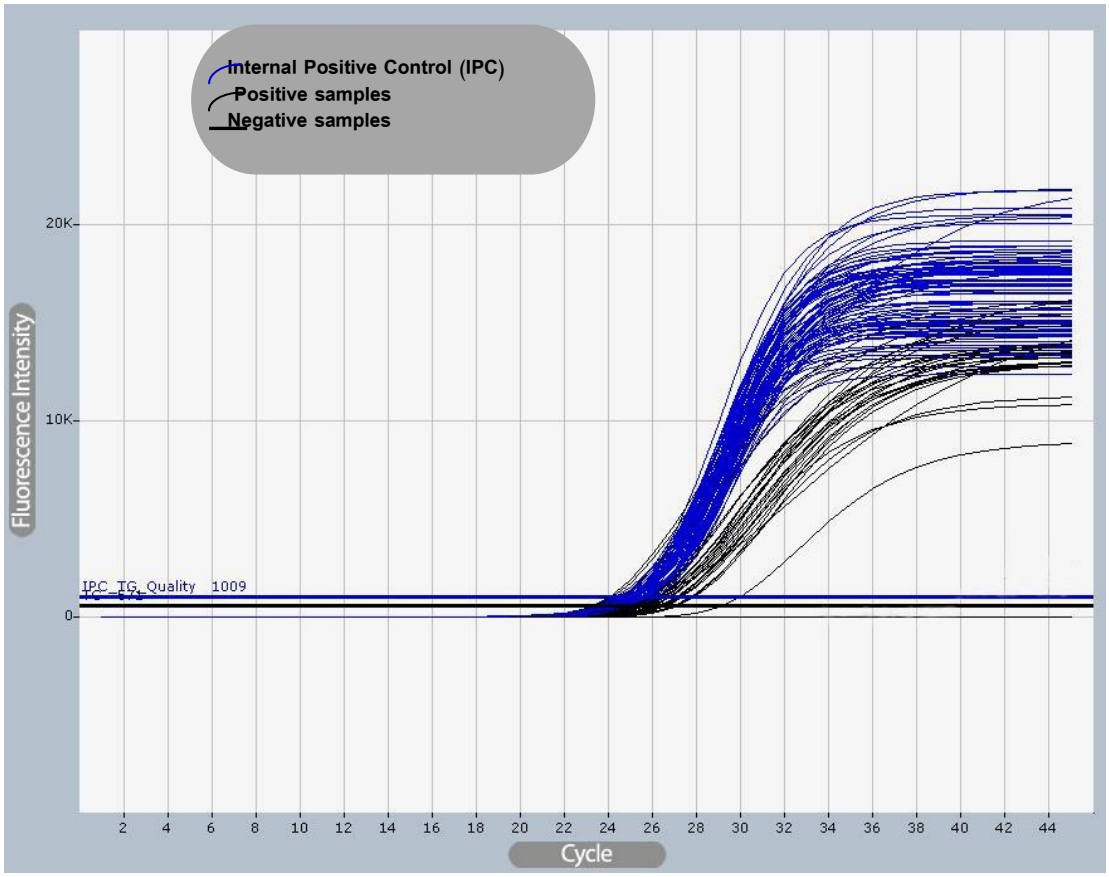


Figure (4): The results of Real time PCR reaction for positive ELISA study groups.