Oncology
and Radiotherapy ©

17 (11) 2023: 001-006 • RESEARCH ARTICLE

Exploring IgA variation in serum and aqueous humor of ocular toxoplasmosis patients: relevance for radiotherapy VMAT strategies

Haneen Ali Kareem, Shatha Khudiar Abbas
[1]Department of Biology/ College of Science / Al-mustansiriyah university, Iraq.
[2]Department of Biology/ College of Science / Al-mustansiriyah university, Iraq.

ABSTRACT

Toxoplasma.gondii is a protozoan eukaryotic parasite that can enter the body host as an obligate intracellular parasite and causes toxoplasmosis infection, it has the ability to cause latent infection in the host tissue such as cardiac muscle, skeletal muscles, or the central nervous system (Mendez et al.2017). Research into T. gondii in cancer patients is extensive. However, no one has yet discussed the implications of widespread parasite infection in this population. (Tarabulsi., 2020).

Ocular toxoplasmosis (OT) is the infection of the eye caused by toxoplasmosis. Toxoplasma gondii infestation, which causes infectious posterior uveitis and consequent vision loss, is a significant health damage, particularly in developing nations (ADEGBEHINGBE, 2020). The study was carried out on 80 sample (50 are patients and 30 as a control) with age range (15-65) years, The serum level (S.IgA) of the group was non-significantly increased in O.T. patients than controls, (107.84 ± 11.1 vs. 93.39 ± 9.67 pg/ml), (P= 0.329) . As well as, the serum level of S.IgA of the group was non-significantly increased in A.T. patients than controls, (100.06 ± 8.03 vs. 93.39 ± 9.67 pg/ml), (P= 0.607) . On the other hand, the serum level of S.IgA of the patients groups was non-significantly increased in O.T. patients than in A.T., (107.84 ± 11.1 vs. 100.06 ± 8.03 pg/ml), (P= 0.573).

The level of Aqueous homer (A.IgA) of the group was significantly increased in O.T. patients than controls, (226.25 ± 12.14 vs. 191.78 ± 9.8 pg/ml), (P= 0.03) . As well as, the level of A.IgA of the group was non-significantly increased in A.T. patients than controls, (198.16 ± 12.4 vs. 191.78 ± 9.8 pg/ml), (P= 0.155) . On the other hand, the level of A.IgA of the patients groups was non-significantly increased in O.T. patients than in A.T., (226.25 ± 12.14 198.16 ± 12.4 pg/ml), (P= 0.112).

Keywords: Toxoplasmosis, O.T., IgA , Uveitis (A.T.) , Aqueous homer, VMAT, Radiotherapy

Address for correspondence:

Haneen Ali Kareem, Department of Biology/ College of Science / Al-mustansiriyah university, Iraq. E-mail: Haneen.ali73@yahoo.com

Word count: 3375 **Tables:** 8 **Figures:** 2 **References:** 28

Received: 06 September, 2023, Manuscript No. OAR-23-115435
Editor assigned: 18 September, 2023, Pre-QC No. OAR-23-115435 (PQ)
Reviewed: 03 October, 2023, QC No. OAR-23-115435 (Q)
Revised: 16 November, 2023, Manuscript No. OAR-23-115435 (R)
Published: 30 November, 2023, Invoice No. J-115435

INTRODUCTION

Ocular Toxoplasmosis (OT) is responsible for the eye infection. Toxoplasma gondii infection, which results in infectious posterior uveitis and vision loss, is a significant health risk, particularly in developing countries.

Toxoplasmosis of the eye is the most common cause of infectious posterior uveitis, and the diagnosis relies heavily on the recognition of characteristic clinical manifestations. Antibody detection in serum or intraocular fluids can be utilized for laboratory confirmation.

Toxoplasma gondii is the most significant protozoan cause of intraocular inflammation due to its global distribution, frequency of ocular involvement, and severity of symptoms. Toxoplasmosis was first identified in congenital disease patients.

Infection in humans can occur either congenitally or acquiredly. After ingestion of oocysts or tissue cysts, acquired disease typically develops, and almost all cases (in immunocompetent individuals) are asymptomatic. Depending on dietary habits, climate, etc., the prevalence of seropositivity due to a previous Toxoplasma infection differs among different populations. By age 40, the majority of the population in the Netherlands has already been infected.

Congenital infection can occur when a pregnant woman is infected; Toxoplasma crosses the placenta and infects the developing embryo during the parasitaemia. Risk and severity of infection for the infant depend on the gestational age of the mother when she contracts the infection.

The severity of a congenital infection is greatest when it is contracted during the first trimester of pregnancy [1-6].

Ocular disease, which is uncommon in such instances, may be atypical, manifesting as bilateral multifocal lesions, for instance.

Patients with AIDS who have (ocular) toxoplasmosis typically require lifelong treatment. Toxoplasmic retinochoroiditis in AIDS is most likely due to (reactivation of) acquired disease, although in some cases it may be caused by congenital ocular abnormalities (Figure 1) [7,8].

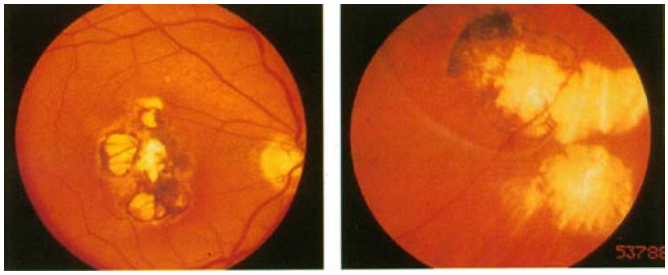

Fig.1 (Left and right) Inactive old toxoplasmosis scar with typical atrophy and hyperpigmentation. Preretinal vitreous detachment is also visible (right).

MATERIAL AND METHOD

The current research was conducted between January / 2022 and February /2023 on 80 samples (50 patients and 30 controls) ranging in age from 15 to 65 years. The current study included 30 participants who appeared to be in good health and whose ages ranged from (20-40) years. That matched the cohort of patients. None of them had any issues, and none of them have any chronic or systemic diseases. The 50 patients are divided into two groups (25 are infected with Ocular Toxoplasmosis (O.T.) and 25 are infected with Uveitis A.T.) based on the results of an ELISA serological test (IgG and IgM) [9].

Sample Collection

Blood collection : Using a 5 ml disposable cannula, approximately 5 ml of human blood was collected intravenously from patient and control groups using a sterile technique.Put in clot activator gel tubes for serum separation and allowed to clot for 30 minutes at room temperature, the sample was centrifuged at 3000rpm for 10 minutes, and the serum was immediately separated into small equal portions in Eppendorf tubes, which will measure levels (IgA ELISA kits) and stored at -20 degrees Celsius until use. Each sample is marked with a serial number and the individual's identity.

Aqueous Homer collection: Aqueous humor was taken under topical anesthesia. All procedures were performed under aseptic conditions in an ocular surgery setting. Briefly, a 31-gauge needle was inserted at the peripheral clear cornea and between 50 and 100µL aqueous humor could be withdrawn under direct control of an operating microscope. All samples were immediately stored and maintained at -50°C to prevent degradation and thawed directly before analysis (IgA) [10-14].

Assay procedure of IgA:

1. Bring all reagents and samples to room temperature (18°C-25°C) naturally for 30min before starting assay procedures.
2. Set Standard wells, Sample wells and Blank/Control wells, added Standard 50µl to each Standard well, add Sample 50µl to each Sample well, add Sample Diluent 50µl to each Blank/Control well.
3. Added 100µl of HRP-conjugate reagent to each well, cover with a Closure Plate Membrane and incubate for 60 minutes at 37°C.
4. Washed the plate 4 times.
5. Added Chromogen Solution A 50µl and Chromogen Solution B 50µl to each well successively. Gently mix and then protect from light to incubate for 15 minutes at 37°C.
6. Added 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Read the Optical Density (OD) at 450 nm using an ELISA reader within 15 minutes after adding Stop Solution.

RESULTS AND DISCUSSION

Tab 1. Serum Levels of S.IgA in O.T. patients and control group.

S.IgA (pg/ml)	**Patient**	**Control**
	(No. = 25)	**(No. = 30)**
Mean ± S.E.	107.84 ± 11.1	93.39 ± 9.67
P-value	0.329 NS	
- NS: Non-Significant.		
S.E: Standard Error, No: Number, *P*: Probability		

Tab 2. Serum Levels of S.IgA in A.T. patients and control group.

S.IgA (pg/ml)	**Patient**	**Control**
	(No. = 25)	**(No. = 30)**
Mean ± S.E.	100.06 ± 8.03	93.39 ± 9.67
P-value	0.607 NS	
- NS: Non-Significant.		
- S.E: Standard Error, No: Number, *P*: Probability.		

Tab 3. Serum Levels of S.IgA in O.T. and A.T. patients groups.

S.IgA (pg/ml)	O.T.	A.T.
	(No. = 25)	(No. = 25)
Mean ± S.E.	107.84 ± 11.1	100.06 ± 8.03
P-value	0.573 NS	
- NS: Non-Significant.		
- S.E: Standard Error, No: Number *P*: Probability.		

(Francis, and Joynson, 1993) demonstrated that Detection of anti–T. gondii IgA antibodies in serum is used to establish recently acquired infection, The low level of IgA in serum supposed to be associated with acute toxoplasmosis (as in date of our research).

IgA in serum is generally replaced by a long-lasting anti–T. gondii IgG response. The presence of intraocular IgA not only during postnatally acquired but also during recurrent disease could be the result of the unique properties of the local environment of the antibody-producing plasma cells.

Francis, and Joynson said in there studied IgA could not be detected until 2 to 4 weeks after onset of symptoms that mean the IgA is always associated with chronic infection (positive correlation with IgG), this study is disagree with our result data as show in Table 1.

Some authors have found that T. gondii IgA testing is controversial and is either too insensitive or reactive for too long, suggesting that IgA is not a dependable marker for a recently acquired Toxoplasma infection. However, similar to the findings reported here, a number of investigators have found that T. gondii IgA is helpful for the diagnosis of recent infection during. These authors detected IgA antibodies directed against P30 in the sera of all patients during the acute phase of toxoplasmosis. when the level of IgG continued to rise and IgM antibodies continue, IgA antibodies earlier disappeared and not detected in the chronic phase of toxoplasmosis. Decoster and Olariu agree with our data . In addition, same testing for T. gondii IgA and IgM antibodies has been marked valuable by other investigators for diagnosing a recently acquired T. gondii infection, especially during pregnancy.

Olariu said the persistence of T. gondii IgA (or IgM) antibodies in chronic infections may be explained patient heterogeneity, infecting parasite stage (oocyst versus cyst), status of immune at the time of blood collection, timeing of blood sampling comparative to the time of onset of infection, assay technique.

The IgA are maybe specific for tachyzoites, or a stage that is an intermediate between T. gondii tachyzoi and bradyzoites-sporozoites.

there are not reports of simultaneous presence of specific IgM, IgA and IgE in reinfections. Then, simultaneous presence of IgM, IgA and IgE can be considered strong evidence for recent primary infection. If this assumption is true, Gómez demonstrated in there studied found that 1/10 (10%) of new cases of ocular toxoplasmosis in Colombian patients from the Quindio region could be related to a recent infection (proportion of cases with specific IgM plus IgA and IgE positive in a first episode). Like Montoya,and Remington, they agreed that finding indicates that recent acquired toxoplasmosis is more frequent than believed.

Specific IgA has been reported in a case of reinfection in women from a French who had an abortion. Similarly, Pinon described that in a group of HIV-infected patients with evidence of previous exposure to T. gondii, but no clinical manifestations, IgA and IgE were detected in 11% and 4% of cases.

Tab 4. Levels of A.IgA in O.T. patients and control group.

A.IgA (pg/ml)	Patient	Control
	(No. = 25)	(No. = 30)
Mean ± S.E.	226.25 ± 12.14	191.78 ± 9.8
P-value	0.03*	
- (*) Significant increase $P < 0.05$ compare to control.		
- S.E: Standard Error, No: Number, *P*: Probability.		

Tab 5. Levels of A.IgA in A.T. patients and control group.

A.IgA (pg/ml)	Patient	Control
	(No. = 25)	(No. = 30)
Mean ± S.E.	198.16 ± 12.4	191.78 ± 9.8
P-value	0.155 NS	
- NS: Non-Significant. - S.E: Standard Error, No: Number, *P*: Probability.		

Tab 6. Levels of A.IgA in O.T. and A.T. patients groups.

A.IgA (pg/ml)	O.T.	A.T.
	(No. = 25)	(No. = 25)
Mean ± S.E.	226.25 ± 12.14	198.16 ± 12.4
P-value	0.112 NS	
- NS: Non-Significant.		
- S.E: Standard Error, No: Number, *P*: Probability.		

Garweg said in there studies that quantitative ELISA, specific IgA, IgM, and IgE types have been demonstrated to occur in ocular toxoplasmosis patients at the following frequencies: IgA, from 26% through 52% to 63%. Labalettein the aqueous humor and no in serum; IgM, from <1% to 11% in the aqueous humor and to 50% in the serum; IgE, from 0% to 14%. Gómez in the vitreal fluid and to 66% in the serum. The levels of IgE within the aqueous humor have not been determined, which is given the purported role of this class of immunoglobulin in ocular toxoplasmosis which related hypersensitivity and autoimmunity. The wide range of sensitivities reported for the different types of specific immunoglobulin probably reflects the usually small number of patients included and the case selection criteria employed, e.g., genetic origin the mode of disease transmission (acquired or congenital) and age.

However, Garweg also said in their studies the indications that the determination of specific IgA within either the aqueous humor or tears may yield information, but unknown specificity or sensitivity. The results of our study as show in Table 4 is probably the result of increased capillary permeability in the inflamed tissues. Immunoblotting revealed the presence of IgA bands in 76% of aqueous humor samples and yielded evidence of local specific IgA production in 35%. This evidence of local specific IgA production was not substantiated in 22% of patients by immunoblot data for IgG or quantitative ELISA. (the Goldmann-Witmer coefficient for IgG and the IgA index). These results are not suggestive of an inhibition or blocking of IgA-specific binding by IgG.

Table 5 show a nonsignificant of A.T. group comparing to control. Martenet disagree with our data, the concentration of IgA in uveitis increases much more than would be expected from its molecular weight, and straightforward passage through the blood-water barrier. While the ratio of IgG to albumin remains nearly constant in normal eyes and those with diverse inflammatory conditions, posterior uveitis is distinguished by an increase in IgA relative to IgG. This relative increase of IgA can also be demonstrated in relation to C3 (complement factors) levels, whereas the concentration of C3 increases in sync with the increase of all aqueous humor proteins.

Aqueous showed some positive IgA in uveitis cases in some patients with ankylosing spondylitis, rheumatoid arthritis, Tuberculosis , Behcet's disease, Idiopathic and syphilis had IgA in their aqueous.

Ghose said that Patients with active uveitis had elevated levels of immunoglobulins in the aqueous humour compared to other patient groups; four out of five patients with active uveitis had detectable IgM in their aqueous humour. Immunoelectrophoretic detected IgG and IgA, but not IgM, in the aqueous humor of the remaining patients with senile cataract and narrow-angle glaucoma.

Differences between S-IgA and A-IgA in OT and AT groups

According to differences between S-IgA and A-IgA in OT patients as shown in Table 7 the results showed that there was significant increase in A-IgA compare to S-IgA (P < 0.001). Table 8 shows the differences between S-IgA and A-IgA in AT patients, the results showed that there was significant increase in A-IgA compare to S-IgA (P < 0.001) (Figure 2).

Tab 7. Differences between S-IgA and A-IgA in OT patients.

OT patients	A-IgA (N=25)	S-IgA (N=25)
Mean ± S.E.	226.25 ± 12.14	107.84 ± 11.11
P-value	< 0.001**	
- Significant at < 0.01.		
- S.E: Standard Error, No: Number, *P*: Probability.		

Tab 8. Differences between S-IgA and A-IgA in AT patients.

AT patients	A-IgA (N=25)	S-IgA (N=25)
Mean ± S.E.	198.16 ± 12.4	100.06 ± 8.03
P-value	< 0.001**	
- Significant at < 0.01.		
- S.E: Standard Error, No: Number, *P*: Probability.		

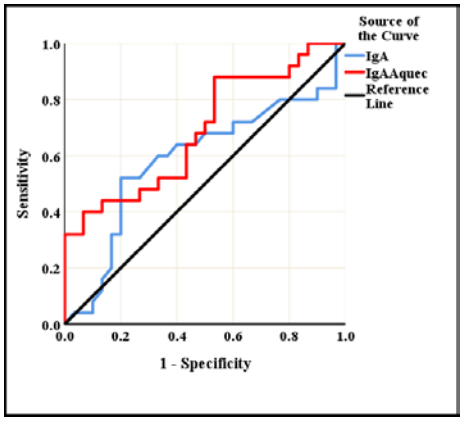

Fig 2. ROC Curve Analysis of S.IgA and A.IgA

CONCLUSIONS

As show in our data the concentration of IgA in Aqueous homer is more significant than in serum, so this supposed to be IgA in Aqueous homer is more specific than IgA in serum to detect ocular toxoplasmosis.

REFERENCES

1. Tarabulsi, M. (Doctoral dissertation, University of Salford).
2. ADEGBEHINGBE, S. A. (Doctoral dissertation, FEDERAL UNIVERSITY OF TECHNOLOGY, AKURE).
3. Kalogeropoulos, D., Sakkas, H., Mohammed, B., et al. (2020). Ocular toxoplasmosis: a review of the current diagnostic and therapeutic approaches. Int Ophthalmol, 42, 295-321.
4. Francis, J. M., & Joynson, D. H. M.. Duration of specific immunoglobulin A antibody following acute toxoplasmosis as determined by enzyme immunoassay and immunosorbent agglutination assay. Eur J Clin Microbiol Infect Dis,1993;12, 556-559.
5. Nascimento, F. S., Suzuki, L. A., & Rossi, C. L. (2008). Assessment of the value of detecting specific IgA antibodies for the diagnosis of a recently acquired primary Toxoplasma infection. Prenat Diagn, 28, 749-752.
6. Olariu, T. R., Blackburn, B. G., Press, C., et al. (2019). Role of toxoplasma IgA as part of a reference panel for the diagnosis of acute toxoplasmosis during pregnancy. J Clin Microbiol, 57, e01357-18.
7. Decoster, A., Caron, A., Darcy, F., & Capron, A. (1988). IgA antibodies against P30 as markers of congenital and acute toxoplasmosis. Lancet, 332, 1104-1107.
8. Antunes Murata, F. H., Ferreira, M. N., Camargo, N. S., et al. (2021). Frequency of anti-Toxoplasma gondii IgA, IgM, and IgG antibodies in high-risk pregnancies, in Brazil. Rev Soc Bras Med Trop, 512-514.
9. Meek, B., Klaren, V. N., van Haeringen, N. J., et al. (1999). IgA antibodies to Toxoplasma gondii in human tears. Investig Ophthalmol Vis Sci, 41, 2584-2590.
10. Montoya, J. G., & Remington, J. S. (1996). Toxoplasmic chorioretinitis in the setting of acute acquired toxoplasmosis. Clin Infect Dis, 23, 277-282.
11. Fortier, B., Aissi, E., Ajana, F., et al. (1991). Spontaneous abortion and reinfection by Toxoplasma gondii. Lancet, 338, 444.
12. Pinon, J. M., Foudrinier, F., Mougeot, G., et al. (1995). Evaluation of risk and diagnostic value of quantitative assays for anti-Toxoplasma gondii Immunoglobulin A (IgA), IgE and IgM and analytical study of specific IgG in immunodeficient patients. J Clin Microbiol, 33, 878-884.
13. Garweg, J. G., Garweg, S. D. L., Flueckiger, F., Jacquier, P., & Boehnke, M. (2004). Aqueous humor and serum immunoblotting for immunoglobulin types G, A, M, and E in cases of human ocular toxoplasmosis. J Clin Microbiol, 42, 4593-4598.
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, 294-300.
15. Labalette, P., Delhaes, L., Margaron, F., et al. (2001). Ocular toxoplasmosis after the fifth decade. Am J Ophthalmol, 133, 506-515.
16. Ongkosuwito JV, Bosch-Driessen EH, Kijlstra A, Rothova A, et al. Serologic evaluation of patients with primary and recurrent ocular toxoplasmosis for evidence of recent infection. Am J Ophthalmol. 1999;128(4):407-412.
17. Liotet S, Bloch-Michel E, Petithory JC, Batellier L, Chaumeil C, et al. Biological modifications of the

vitreous in intraocular parasitosis: preliminary study. Int Ophthalmol. 1992; 16:75-80.
18. Nussenblatt RB, Mittal KK, Fuhrman S, Sharma SD, Palestine AG, et al. Lymphocyte proliferative responses of patients with ocular toxoplasmosis to parasite and retinal antigens. Am J Ophthalmol. 1989;107(6):632-641.
19. Villena ID, Aubert D, Brodard V, Quereux C, Leroux B, et al. Detection of specific immunoglobulin E during maternal, fetal, and congenital toxoplasmosis. J Clin Microbiol. 1999;37(11):3487-3490.
20. Loyola AM, Durighetto Jr AF, Silva DAO, Mineo JR, et al. Anti-Toxoplasma gondii immunoglobulins A and G in human saliva and serum. J Oral Pathol Med. 1997;26(4):187-191.
21. Ghose T, Quigley JH, Landrigan PL, Asif A, et al. Immunoglobulins in aqueous humour and iris from patients with endogenous uveitis and patients with cataract. Br J Ophthalmol. 1973;57(12):897.
22. Martenet AC, Kälin A, Grob PJ, et al. Newer immunological studies of the aqueous humor in Uveitis. Ophthalmic Res. 1980;12(2):111-117.
23. Lynch MI, Malagueño E, Lynch LF, Ferreira S, Stheling R, et al. Anti-Toxoplasma gondii secretory IgA in tears of patients with ocular toxoplasmosis: immunodiagnostic validation by ELISA. Mem Inst Oswaldo Cruz. 2009;104:818-822.
24. Gómez-Marín JE, Montoya-de-Londoño MT, Castaño-Osorio JC, Heine FA, Duque AM, et al. Frequency of specific anti-Toxoplasma gondii IgM, IgA and IgE in Colombian patients with acute and chronic ocular toxoplasmosis. Mem Inst Oswaldo Cruz. 2000;95:89-94.
25. Greigert V, Bittich-Fahmi F, Pfaff AW, et al. Pathophysiology of ocular toxoplasmosis: Facts and open questions. PLoS Negl Trop Dis. 2020;14(12):e0008905.
26. Mendez OA, & Koshy AA. Toxoplasma gondii: Entry, association, and physiological influence on the central nervous system. PLoS Pathog. 2017;13(7):e1006351.
27. Alsailawi HA, Ayat SS, Mudhafar M, Mohammed RK, Dhahi SJ, et al. Immunohistochemical study of TGF-β, PDL-1, and IL-10 in patients with Toxoplasma gondii. World J Adv Res Rev. 2022;14(1):436-448.
28. Cochereau-Massin I, LeHoang P, Lautier-Frau M, Zerdoun E, Zazoun L, et al. Ocular toxoplasmosis in human immunodeficiency virus-infected patients. Am J Ophthalmol. 1992;114(2):130-135.