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Seroprevalence of Toxoplasma Antibody and Clinical Features of Toxoplasmosis in Posterior Uveitis in South-West Nigeria

Oluwatoni O. Onabolu[1*], Olatunbosun Olawale[2], Olubunmi T. Bodunde[1], Adetunji H. Ajibode[1], Taibat O. Otulana[1], Olubukola A. Ayeni[1], Adeduntan S. Olagbenro[1] and Philip Olatunji[3]

[1]Department of Ophthalmology, Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria.
[2]Department of Chemical Pathology, Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria.
[3]Department of Haematology, Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OO, PO, OAA and ASO managed the analyses of the study. Authors OTB, AHA and TOO managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Toxoplasmosis is the presumed cause of posterior uveitis in South West Nigeria, and patients are mostly treated empirically on the basis of clinical findings.
Aim: To determine the level of IgG and IgM toxoplasma antibodies consistent with diagnosis of toxoplasma associated posterior uveitis in immune-competent subjects and compare with normal controls.
Design: A cross-section analytic study.

**Corresponding author: E-mail: oluwatonionabolu@gmaill.com, oluwatonionabolu@gmail.com, tonionabolu@yahoo.com;*

Place and Duration: Department of Ophthalmology, Olabisi Onabanjo University Teaching Hospital Sagamu Ogun State from July 2016-March 2017
Methodology: Consecutive patients with clinical signs of posterior uveitis were recruited and had questionnaires administered to obtain socio-demographic data and risks of toxoplasma infection. Venous blood was collected for hemoglobin concentration, white blood cell count differentials, HIV seropositivity, and for ELISA immunoglobulin assay for Toxoplasma IgG and IgM antibodies.
Results: Thirty-four consecutive cases with a clinical diagnosis of posterior uveitis and nineteen age sex-matched healthy controls were recruited for the study. Twenty-five (73.53%) cases had significantly elevated levels of Toxoplasma IgG and 3 for IgM (8.82%); while amongst the controls, 31.6% had significantly raised IgG while 15.8% had for IgM. The difference between cases and controls for elevated serum levels of Toxoplasma antibodies was statistically significant (p=.003).
Conclusion: Elevated serum IgG toxoplasma antibodies are useful in the diagnosis of posterior uveitis caused by *Toxoplasma gondii* and fairly corresponded to the clinical fundus finding.

Keywords: Ocular toxoplasmosis; posterior uveitis; igg seropositivity.

1. INTRODUCTION

Toxoplasmosis is the most common cause of posterior uveitis in immunocompetent persons worldwide [1,2]. The causative organism is the parasite *Toxoplasma gondii* a protozoan whose primary host is the cat. Man is primarily infected through oral ingestion of toxoplasma oocysts produced in the intestine and excreted through the faeces of its specific host the cat and other felids [3]. Secondly by oral uptake of toxoplasma cysts which persist in the skeletal muscles of pigs and sheep [3]. Thirdly through drinking of infected water and eating infected mussels [4]. The success of infection caused by *T.gondii* is based on a delicate balance between the host immune response which tries to clear the parasite and the immune evasion strategy or immunomodulation elicited by the parasite which enables the ultimate survival of the parasite and the host [5].

Earlier studies reported that acute toxoplasma chorioretinitis in adults is a late manifestation of congenital infection [6]. However, recent evidence may suggest that most individuals were infected postnatally [6]. Acquired infections are asymptomatic or produce flu-like symptoms [7]. After ingestion of the cyst, tachyzoites enter the bloodstream through the intestinal wall. Cell-mediated tissue response converts tachyzoites into bradyzoites. They are then carried to all organs and become established as cysts containing bradyzoites [8].The host defense mechanism produces immunoglobulin against the parasites. Recent infections stimulate the production of IgM antibodies which is later replaced by IgG. While IgM is transiently detectable early in the disease, IgG persists throughout life [9]. Given a high prevalence of *T.gondi* antibodies in most communities, serologic testing is generally not useful; therefore clinical fundus findings of fluffy white areas of retino-choroiditis adjacent to retinal scars are presumed to be diagnostic [1]. However, laboratory studies have shown that high levels of IgG, IgA, and IgG avidity tests can be used for definitive diagnoses [9-11]. This is necessary in atypical clinical findings and in pregnant women to prognosticate the effect on the foetus [1]. Other laboratory methods of diagnosing ocular toxoplasmosis include Toxoplasma dye test, Polymerase chain reaction, Fluorescent Test and using synthetic chemicals [12-17]. Two or more tests may be required for definitive diagnosis [12]. Recently recombinant antigens are used to increase specificity [18]. In low-income countries, the laboratory tests are rarely done although expert interpretation of photographs for toxoplasmosis was not always reliable [19-21]. However, prevalence studies for IgG antibodies in these countries revealed varying levels of seropositivity to toxoplasmosis supporting this diagnostic assumption [22-24]. Toxoplasmosis is a significant cause of ocular morbidity and blindness especially in young adults [25]. This study aims to document the elevation of IgG and IgM antibodies to *Toxoplasma gondii* in competent immune patients with clinical diagnosis of toxoplasma posterior uveitis in a Nigerian community.

2. METHODOLOGY

2.1 Study Design

A cross-sectional analytic study of patients with toxoplasmosis to determine the level of IgG and IgM.

2.2 Study Location

The study was carried out at the department of Ophthalmology, Olabisi Onabanjo University Teaching Hospital Sagamu Ogun State from July 2016-March 2017.

2.3 Case Definition

A case of Toxoplasmosis is defined as a case of posterior uveitis with focal necrotizing retino-choroiditis with or without an anterior spill or an eye with dense vitritis precluding clear view of the retina. Age sex-matched controls were patients booked for refraction with no other ocular or systemic disease. Presenting visual acuity ≤ 6/18-6/60 was taken as visual impairment and < 3/60 with glasses correction as blind.

2.4 Questionnaire Administration, Clinical Examination and Blood Investigation

Consecutive patients with clinical signs of posterior uveitis were subjected to interviewer administered questionnaire to obtain personal information on bio data and toxoplasmosis infection risks, onset of visual symptoms, systemic symptoms followed by ocular clinical examination. This consisted of best corrected visual acuity (Snellen's Literate and Tumblin E), slit lamp examination (with Haag-Streit 900), intra ocular pressure with Goldman's applanation tonometer on a slit lamp and dilated fundoscopy using direct and indirect ophthalmoscopes in both eyes. Age and sex matched controls were also subjected to dilated fundoscopy to exclude retinal abnormalities especially retinal scars. Venous blood was taken from subjects and age sex matched normal controls for Haemoglobin (Hb), White blood cell count (WBC) and differentials, retroviral test for Human Immunodeficiency Virus (HIV) with Determine kit HIV ½ (Alere Medical company Ltd Japan)and Enzyme linked immunosorbent assay (ELISA) for Toxoplasma IgG and IgM antibodies (GENWAY BIOTECH USA).

The clotted blood for ELISA was centrifuged at 3,000g for 10 mins after which the serum was separated and stored in the freezer at -20^{o}C to -70^{o}C. The blood samples were analyzed for IgG and IgM using the ELISA kit manufactured by GENWAY BIOTECH Inc San Diego CA92121USA (Product number: 40-521-475131). Before assaying, all samples were diluted 1+100 with IgG sample diluent. Then the company protocol was strictly adhered to. The kit quantitatively measured the levels of Toxoplasma IgG and IgM in the sera samples. The value of IgG>50 IU was positive while IgM > 10 IU/Ml was positive. Subjects who were positive for HIV were excluded from the study but treated for the infection. Informed written consents were obtained from subjects and controls. The study was approved by the Ethical committee of OOUTH. Cases with posterior uveitis were treated appropriately according to the hospital's protocol. The study adhered strictly to the tenets of Helsinki concerning human research.

2.5 Data Analysis

Results of the toxoplasma IgG, IgM, and clinical data were inputted into Statistical Package for Social Sciences IBM version 20 spreadsheet in computer and comparative analysis was done. Discrete variables were compared using chi-square and Odds Ratio. A significant difference was determined at p value < 0.05. Continuous variable was compared using arithmetical proportions.

3. RESULTS

Thirty-six subjects were seen from July 2016 to March 2017 with a diagnosis of posterior uveitis. Two subjects who were retroviral positive were excluded. The subjects included 20 males and 14 females (m:f ratio 2:1.4). The mean age of the subjects was 31.6±10.8 (range 18-59 years), while that of controls was 33.0±12.7 (range 18-59 years). Of the 34, the two eyes of four patients were affected, while one eye each was affected in 30 patients. Therefore 38 eyes of 34 patients and 19 age sex matched controls were analyzed. The most commonly affected age group was 20-39 years consisting of 22 (64.7%) subjects. The clinical features are shown in Table 1. Majority (88.2%) were unilateral. Almost half (47%) presented within 14 days of commencement of symptoms with 17.7% presenting after a month. All the cases presented with cloudy vision. A majority (76.5%) had no previous attacks. Majority also (67.65%) had plain posterior uveitis, whilst the remaining had pan uveitis. Retinal scars associated with recent retino-choroiditis were seen in 19 eyes, 5 of which were on the macular. In 5 eyes the severe vitritis precluded further view of the retina. No retinal scars were found in 39.47% of eyes. Vasculitis and retinal haemorrhages were noted as shown (Table 1). The intraocular pressure

remained within normal limits between 10 and 21 mmHg, except in one patient who developed secondary glaucoma after occlusio pupillae. Other complications were cataracts in 23.68% of eyes, and retinal detachment in one eye (2.6%).

Table 2 showed the mean of IgG seroprevalence in each age group. Twenty five (73.5%) subjects and 6 controls were seropositive to Toxoplasma IgG.

Two of the cases with seropositivity for toxoplasmosis had pan uveitis and the rest posterior uveitis. In the cases the range of IgG was from 3.170 IU/mL to 312.222 IU/mL with a mean of 142.589 IU/ml (SD 83.450). In the control it was from 2.613 IU/ml to 302.091 IU/ml with a mean of 66.738 IU/ml (SD 92.540). The difference in the seropositivity for IgG in the controls and cases was statistically significant p=0.003 (X^2 8.835 df 1, *p value* .003 < than 0.05). Patients with vitritis were more likely to have a positive IgG and this was statistically significant p<0.05, Fishers Exact p=0.009328. The sensitivity of IgG test was 80.65% while the specificity was 59.09% in this study.

Three (8.8%) cases were seropositive for IgM. All three had severe vitritis. They were younger in age and they were also positive for IgG. Three controls (15.8%) were positive for IgM (Table 2).

Table 1. Clinical presentation of cases of toxoplasmosis

Clinical features	Number of patients/eyes	Percentage (%)
Laterality		
Unilateral	30	88.2
Bilateral	4	11.8
Duration of symptoms		
3 to 14 days	16	47
14 days to 1 month	12	35.3
➢ 1 month	6	17.7
Previous attacks		
Yes	8	23.5
No	26	76.5
Anatomical involvement		
Posterior uveitis	23	67.6
Panuveitis	11	32.4
Posterior segment signs (Eyes)		
Retinal scar + active lesion	19	50
Active lesion + no scar	14	36.8
Severe vitritis	5	13.2
Additional clinical findings(Eyes)		
Retinal vasculitis	10	26.3
Retinal haemorrhages	2	5.3
Cataracts	9	23.7
Raised IOP	1	2.6
Retinal detachment	1	2.6

Table 2. Levels of IgG in cases and controls

		IgG levels of subjects		IgG levels of controls
Age group in years	**N1**	**Mean±SD**	**N2**	**Mean±SD**
0-19	4	151.5 ±97.0	4	54.7 ±96.9
20-39	22	139.8±86.3	10	25.6 ±61.3
40-59	8	145.7±79.8	5	158.6 ±90.0
Total	34	142.6 ±83.5	19	66.7 ±92.5

Key: N1; Subjects
N2: Controls

The mean white cell counts were 5.5±2.3 X 10^9/L and 5.8±1.3 X 10^9/L in cases and controls, respectively, while the lymphocyte counts were 2.6±0.8 X 10^9/L, and 2.9±0.7 X 10^9/L, respectively. There were no statistically significant differences.

Twenty (52.6%) eyes of subjects (cases) with posterior uveitis were visually impaired, 14 (41.2%) were blind, while 4 eyes were not visually impaired. Post treatment, vision improved in ten eyes (26.31%) while 12 (35.3%) remained blind. Table 3 showed the pre and post treatment visual acuity of the cases. Ten cases with 10 visually impaired eyes were lost to follow up.

4. DISCUSSION

Ocular toxoplasmosis in this study was most common in the 20-39 years age group in support of the findings that ocular toxoplasmosis manifests in the second through the fourth decades of life . [1,7]. Ayanru in Nigeria also reported a preponderance of uveitis between 19 and 29 years [19] similar to the findings of Jones et al in Brazil but in contrast to Abraham in Uyo and Kameni in Maiduguri (Nigeria) who reported most common age group to be 41-50 years and 51- 60 years respectively [26,27,23]. There was a male preponderance in our study similar to the report of Jones, Uneke and Kamani but in contrast to Abraham who reported a female preponderance [26,22,23,27]. The male preponderance could be because males are more in contact with animals and soil.

This study showed that 73.53% of subjects and 31.5% of controls were seropositive for IgG. This difference was statistically significant. This result corroborated the report of several authors that *Toxoplasma gondii* is the most common cause of posterior uveitis in immune-competent persons and that ELISA immunoglobulin test can be used for diagnosis especially in doubtful cases [1,3,6,10]. Thus, the clinical finding of acute retino-choroidal lesions correlated with the laboratory results in over 73% of the patients supporting a diagnosis of toxoplasmosis where laboratory investigation cannot be done. However laboratory tests are important in atypical fundus findings especially in patients with acquired immunodeficiency syndrome and in pregnancy to forestall infection of the foetus for early treatment [1].

Significant IgM titre is dependent on the time of infection which is difficult to determine due to late presentation in our patients. Indeed the three cases that were positive for IgM were seen within three weeks of symptoms supporting early production of IgM [8,10].

Seropositivity in the controls (36.8%) shows previous infections and lifelong presence of antibodies against *T.gondii* [1]. The IgG test was found to be sensitive but with less specificity in this study for uveitis.

Unilateral ocular toxoplasmosis was more common than bilateral cases in this study affecting 30 (88.2%) patients in agreement with previous research [1]. Bilaterality was more in the younger age group with vision and clinical severity worse in one eye. Vitritis accompanying posterior uveitis in this study was common in agreement with the Korean study and in contrast to the Serbian study [28,29]. It was found in this study that the severity of vitritis portends the severity of the disease and was significantly related to seropositivity.

The presence of retinal scars indicated previous attacks. However despite the presence of such scars in 19 patients only, 8 admitted to have had previous blurring of vision which cleared without treatment. It is possible that the other 15 eyes of15 patients with no previous scars are recently acquired infections yet as expected IgM antibodies was not positive in all of them

Table 3. Pre and 2 months post treatment visual acuity of 38 eyes with ocular toxoplasmosis

Pre-treatment		Post-treatment visual acuity	No of eyes
Visual acuity	**No. of eyes**		
6/9-6/12	4	6/6-6/12	14
6/18-6/60	20	6/18-6/60	2
3/60-NPL	14	3/60-NPL	12
		Lost to follow up	10
Total	38	Total	38

probably because they did not present early within fourteen days of symptoms [8]. Conversely not all patients with retino- choroidal scars and typical clinical findings were positive with ELISA test in this study. This also confirms the low specificity of ELISA test alone. The five patients with macular scars probably had congenital lesions [25].

There were no significant differences in the total white cell count or lymphocyte count between cases with posterior uveitis and controls which should have been expected in acute/chronic infection. Leucocyte count is therefore not useful in suspecting association of toxoplasmosis with posterior uveitis in our environment.

Vision was adversely affected in patients with posterior uveitis in this study confirming that ocular toxoplasmosis is a significant cause of ocular morbidity and vision loss [25]. Even after treatment, 12 eyes remained blind. This was due in part to secondary cataracts, macular scars, occlusion-pupillae, and retinal detachment. There was no change in the intraocular pressure except in anterior uveitis spill complicated by occlusion-pupillae.

5. CONCLUSION

Elevated IgG toxoplasma antibodies are useful in the diagnosis of posterior uveitis caused by *Toxoplasma gondii* and correspond with clinical diagnoses in most cases. Lymphocyte count does not contribute to diagnostic suspicion.

CONSENT

Written consent obtained from participants.

ETHICAL APPROVAL

Ethical approval was obtained from Ethical Committee of Olabisi Onabanjo University Teaching Hospital.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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