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DEMONSTRATION OF LOCAL ANTITOXOPLASMIC IMMUNOGLOBULIN G PRODUCTION IN OCULAR TOXOPLASMOSIS, TRACED BY ENZYME IMMUNOASSAY OF AQUEOUS HUMOR AND SERUM

M. KEYHANI, M.D., Ph.D., H. SABERI,* M.D., AND H. AHMADIEH, M.D.**

*From the Department of Biochemistry, Iran University of Medical Sciences, the *Department of Neurosurgery, Tehran University of Medical Sciences, and the **Department of Ophthalmology, Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran.*

ABSTRACT

Forty patients with cataracts, as well as 40 patients with proven ocular toxoplasmosis were enrolled in this prospective clinical study. Serum IgG and aqueous IgG in both groups were measured by enzyme-linked immunosorbent assay (ELISA) and the corresponding ratios were calculated. Serum IgG/aqueous humor IgG ratio was less than 100 in the patient group and more than 100 in the control group. On the other hand in the chorioretinitis subgroup the ratio was less than 13, while in the group with uveitis, the ratio was more than 13 ($p<0.05$). The results revealed that calculating the ratio of *Serum IgG (antitoxo) / Aqueous IgG (antitoxo)* may be helpful as an adjunct to diagnosis in cases with clinically atypical ocular toxoplasmosis.

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INTRODUCTION

Toxoplasma gondii, an obligate intracellular protozoon that is ubiquitous in nature, is an important cause of infection and disease in humans and animals.[1] Recurrent ocular toxoplasmosis is the most frequent cause of posterior uveitis and is an important cause of blindness throughout the world.[7]

A confirmed diagnosis of ocular toxoplasmosis indicates the need for extended ophthalmic supervision, with prompt institution of specific treatment in acute exacerbations if indicated. The toxic effects of antiparasitic agents employed are well recognized, consequently there is a need to establish a definite diagnosis of ocular toxoplasmosis as early as possible.[5] In a number of patients the clinical picture may be atypical or fundoscopy may not be possible due to inflammatory response in the vitreous, thus various other causes should be considered in the differential diagnosis. The presence of circulatory anti-toxoplasmal antibody is however not of great importance in view of the fact that many healthy individuals also have these antibodies resulting in a less specific diagnostic value. The absence of circulating antibody on the other hand makes the diagnosis of ocular toxoplasmosis unlikely.[1]

In patients with detectable serum anti-toxoplasma antibody, a suspected toxoplasmal chorioretinitis can be confirmed by measurement of intraocular antitoxoplasmal antibody titers and obtaining the serum IgG/aqueous IgG ratio.[12] This study was undertaken to elucidate the role of local antibody measurements in the diagnosis of ocular toxoplasmosis which may especially be helpful in cases with atypical retinal lesions.

Correspondence: Mahindokht Keyhani, M.D., Ph.D., Department of Biochemistry, Iran University of Medical Sciences, Tehran, P.O. Box: 14155-6183, I.R. Iran.

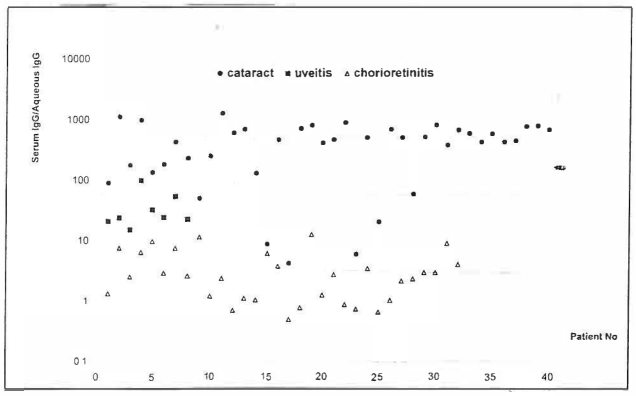

Fig. 1. Serum IgG/aqueous IgG ratio in the cataract group and the patient group including the subgroups of uveitis and chorioretinitis.

PATIENTS AND METHODS

The studied population was comprised of 40 clinically normal subjects undergoing operation for cataract and 40 patients with clinically proven ocular toxoplasmosis. The latter group was further comprised of 32 cases of acute chorioretinitis without uveitis and 8 patients having chorioretinitis and acute uveitis, the two groups being matched regarding age and sex. The diagnosis was based on the characteristic morphologic appearance of retinal lesions and all patients suffered from acute exacerbations of visual symptoms.

Venous blood samples were taken from all patients, then 200-300 μL of aqueous humor was aspirated through inferior limbal puncture with an insulin syringe inserted with an acute angle by the operating ophthalmologist. None of the aqueous samples were allowed to be contaminated by blood. The sera and liquids were kept at -20°C until the testing date utilizing the antitoxoplasmic kit and automatic enzyme linked immunosorbent assay (ELISA) apparatus (Behring ™) in 492 nm wave length.

The positive control serum and aqueous samples were diluted up to 1/100 and 1/10 respectively. The IgG level was calculated by the following formula and measured in international units (IU).[12]

$$\text{Antibody Level} = \frac{OD\ sample - OD\ negative\ control}{OD\ positive\ control - OD\ negative\ control}$$

At the next stage employing the ensuing formula:

$$\text{Ratio} = \frac{Serum\ IgG}{Aqueous\ IgG}$$

the "ratio" was calculated for each patient. A ratio less than 100 was assumed to be a positive result. The two groups were compared with respect to the calculated ratios.

RESULTS

In 33/40 control cataract patients the deduced ratio of serum antitoxoplasma IgG to aqueous antitoxoplasma IgG was more than 100 while in 39 patients of the second group with ocular toxoplasmosis this ratio was less than 100 (Fig. 1). On the other hand in 32/32 patients with chorioretinitis, the ratio was less than 13, while in 8/8 patients with toxoplasma uveitis the ratio was more than 13 and in 7/8 cases less than 100 (Fig. 1). Statistical characteristics of each group are tabulated in Table I. The mean ratios differed significantly between the three groups ($p<0.05$).

Table I. Patient characteristics in the studied groups.

Group	Disease	Number	*Ratio (mean±SD)
Control	Cataract	40	486 ± 326
Patient	Chorioretinitis	32	3.7 ± 3.4
Patient	Uveitis	8	37 ± 29

*Ratio= Serum Ig G / Aqueous Ig G

DISCUSSION

Toxoplasma gondii infection is an important cause of uveitis and chorioretinitis. Most cases of toxoplasma infection result from congenital infection.[7] Patients are often asymptomatic during life, with a peak incidence of symptomatic illness in the second and third decades. Diagnosis is mainly supported by ophthalmological examination and a good response to initiated therapy; however, establishment of a diagnosis by ophthalmological examination alone can be difficult in some cases.[6] Toxoplasma chorioretinitis is probably excluded when serologic tests for IgG antibody yield negative results performed on undiluted serum. When the retinal lesion is characteristic and the serologic titer is positive, most authorities consider that toxoplasma chorioretinitis can be diagnosed with confidence. However, when the retinal lesion is atypical and the serologic titer is positive, the diagnosis of toxoplasma chorioretinitis is only presumptive, because the high prevalence of antibodies in the healthy population precludes the assumption of a causal relationship. Thus serological examination has been useful as an adjunct; however, shortcomings force one to develop more sophisticated methods to delete the false positive results due to life long exposure and high chance for seropositivity.[1]

Enzyme immunoassay has been used for various types of immunoglobulins[11] and cytokines,[10] and polymerase chain reaction (PCR) has been employed for detection of toxoplasma antigens[4] in aqueous humor and vitrectomy

fluid.[9] We also applied enzyme immunoassay for detection of IgG in aqueous humor. Although total IgG has been measured in many studies,[5] it was not required in the serum IgG/aqueous IgG ratio which was used in our study. However, the false positive and false negative results remained in an acceptable range.

The above mentioned ratio was less than 100 in the patient group and more than 100 in control cases, while the classic Goldman-Witmer coefficient which requires total IgG measurements has been reported to be significant if it is more than 8.[1,6]

Goldman Witmer Coefficient=

$$\frac{\text{Antibody Titer Aqueous}}{\text{Total Immunoglobulin Aqueous}} \Big/ \frac{\text{Antibody Titer Serum}}{\text{Total Immunoglobulin Serum}}$$

Therefore when total IgG is not measurable due to small volume of the aqueous sample, the ratio of serum IgG/aqueous IgG can be helpful and reliable.[12] On the other hand we were able to differentiate posterior uveitis from more anterior involvement, that is, the ratio was less than 13 in patients with chorioretinitis and more than 13 in those having toxoplasma uveitis.

In AIDS patients due to suppression of the immune reponse and overwhelming infection, the antibody ratio shall not be helpful, while PCR has been more reliable because of the abundance of parasitic antigens within the aqueous humor.[2] None of our patients had immune deficiency to address this feature.

Recent studies however show more promising results in cats[8] and humans[2,3] with acombination of aqueous PCR and antibody ratio measurment. Although in the PCR technique the toxoplasma DNA is directly detected, its major drawback is lack of widespread availability. This test was not performed in our cases.

Regarding our results obtained from aqueous humor analysis in ocular toxoplasmosis, we recommend performing aqueous humor sampling and IgG measurement in immunocompetent patients with suspected ocular toxoplasmosis with nonspecific funduscopic findings or invisible fundus due to haziness of the ophthalmic media to promote timely and correct diagnosis.

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