Estimation of anti-Toxoplasma Antibodies and Toxo-IgG Avidity in The Tear Film as a Diagnostic Tool for Early Detection of Human Ocular Toxoplasmosis

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Objective: This study was designed to ascertain the diagnosis of active human ocular toxoplasmosis by detection of local anti-Toxoplasma IgA, IgM and IgG, as well as Toxo IgG avidity in tear samples. Subjects and methods: Patients attending the Outpatient Clinic of Ophthalmology Department–Tanta University Hospital during the period from April 2005 to June 2006 were examined and those suspected to have ocular lesions compatible with ocular toxoplasmosis were selected. The patients were classified into two groups: Group (I) : 14 patients with signs of active posterior uveitis presumably due to toxoplasmosis (APUPT). Group (II): 26 patients with chronic retinochoroidal lesion without active uveitis. A third group of 20 healthy individuals were chosen as a control group (group III). Tears and serum samples were collected and were investigated for anti-Toxoplasma IgA, IgM and IgG. The positive samples for anti-Toxoplasma IgG were further investigated for Toxo-IgG avidity. Results: Regarding group (I) with APUPT, it was found that the tears of 9 out of 14 examined patients (64%) showed secretory IgA (sIgA). In addition, 10 out of 14 patients (71%) had positive IgG values in their tears, nine of them had low avidity IgG, with only one patient had high avidity IgG. The simultaneous presence of sIgA and low avidity IgG in the tears denoted recent active infection. The serum samples showed the detection of IgA in 5 patients (36%) and IgG in 8 patients (57%). In group II, sIgA could be detected in tear samples of 2 out of 26 patients (8%), whereas 19 patients (73%) had positive IgG values that all were high avidity. In serum samples, there were 3 patients (12%) with positive IgA, while 21 patients (81%) had positive IgG that all had high avidity. In the control group (III), only one subject out of 20 controls (5%) had IgA in both tear and serum samples. There were 4 and 7 patients (20% and 35%) presenting IgG in tear and serum samples respectively. All IgG had high avidity in both tear and serum samples. Anti-Toxoplasma IgM antibodies were not detected in any of tear or serum samples of all subjects in all groups. Conclusion: From this study it can be concluded that the simultaneous detection of anti-Toxoplasma sIgA and low avidity IgG in tear samples could be considered as a useful marker for early diagnosis of active ocular toxoplasmosis. Moreover, the tear fluid can be considered as a promising candidate for detection of local ocular antibodies because its collection is simple, easy and noninvasive.

INTRODUCTION

Toxoplasmosis is a worldwide infection caused by the obligatory intracellular protozoan Toxoplasma gondii. The infection is usually acquired by ingestion of undercooked meat infected with Toxoplasma cysts or by ingestion of food and drink contaminated with oocysts passing with the stool of infected cats. Acute acquired infection in immunocompetent individuals is usually asymptomatic and spontaneous recovery is the rule. However, 10 to 20% of patients with acute infection may develop cervical lymphadenopathy and/or a flu-like illness. In immunocompromised patients, toxoplasmosis is always due to reactivation of a latent infection[1]. Congenital toxoplasmosis results from an acute primary infection acquired by the mother during pregnancy. The infection constitutes great hazards to the mother and her fetus due to transplacental transmission. In early gestation, the infection may result in miscarriage, stillbirth or premature labour. Maternal infection during the second trimester leads to the birth of infected infants with classical signs of congenital toxoplasmosis that include retinochoroiditis, hydrocephalus and brain calcification. Infection during the third trimester gives birth to infants with subclinical infection who will subsequently develop signs of toxoplasmosis particularly ocular affection in the second or third decade of life[2]. Ocular toxoplasmosis is a local manifestation of systemic infection in which
Toxoplasma spreads into the eye affecting mainly the posterior segment. The hallmark of ocular toxoplasmosis is a necrotizing retinochoroiditis, which has been found to be the most recognizable cause of posterior uveitis (intraocular inflammatory syndrome)\(^3\). Ocular involvement in toxoplasmosis has been mainly considered a recurrent manifestation of congenital infection. However, acquired Toxoplasma infection in adults rarely progresses to retinochoroiditis that is generally unilateral\(^4\). In congenital Toxoplasma infection, retinochoroiditis develops gradually in weeks to years after subclinical congenital infection. This is the predominant form which is frequently bilateral. Ocular lesions may be active or quiescent and scarred. Active ocular lesions consist of well-defined foci of granulomatous retinochoroiditis with necrosis, unique or multifocal, and may be associated with other preexistent lesions. Lesions heal by scar formation that shows well-limited borders with variable degrees of pigmentary epithelium hypertyrophy, retinochoroidal atrophy with occasional vasculitis, hemorrhage and vitreitis\(^5\). Once a scar is established, it remains for the whole life. Recurrence lesions around scars are common and can aggravate the visual deficiency with the possibility of loss of visual function particularly when the macula region is involved\(^6\).

Serological tests are the main tool for laboratory diagnosis of toxoplasmosis. In recently acquired Toxoplasma infection, IgG antibodies rise one week after, which are low avidity antibodies. Later, IgG antibody titers decrease and high avidity IgG may persist for years with absence of IgM. Increased serum IgM and IgA has been found in recent primary infection. The reactivation of toxoplasmic infection, leads to a secondary immune response with IgG production\(^7\).

In ocular toxoplasmosis, the value of serological diagnosis is limited, as IgG is usually present in low concentration in the serum while IgM is absent\(^8\). The laboratory diagnosis of ocular toxoplasmosis could be confirmed by the simultaneous detection of anti-Toxoplasma antibodies in both serum and intraocular fluids as aqueous humor and vitreous\(^9\). However, the collection of aqueous humor or vitreous samples is dangerous due to the possible complications of puncturing the eye. It has been shown that anti-Toxoplasma antibodies (IgG, sIgA IgD and IgE) could be detected also in the tear film that constitutes a part of mucosal immune reponses in ocular toxoplasmosis. Therefore, tears are considered as a possible candidate to investigate the presence of local specific anti-Toxoplasma antibodies in ocular toxoplasmosis\(^10\).

The detection of local secretory IgA (sIgA) in tears of patients with ocular toxoplasmosis could be explained on the basis that the tear film constitutes the first defensive barrier of the eye to infectious agents as it contains sIgA together with active phagocytes and lysozymes\(^11\). Since the gastrointestinal tract is the portal of entry of the parasite, so the first immunologic barrier encountered by the parasite is the mucosal immune system\(^12\). The humoral component of this barrier is represented by sIgA, which interferes with the ability of pathogenic microorganisms, such as T. gondii, to adhere to the mucosal surface, thereby preventing systemic infection. Moreover, it has been demonstrated that protective anti-T. gondii sIgA is present in whey of the breast milk of acutely infected and chronically infected women, indicating that acute infection indeed coincides with a common mucosal immune response\(^13\).

The measurement of Toxo IgG avidity (functional affinity) is a highly useful marker for discrimination between old and recently acquired toxoplasmosis. The avidity of specific IgG denotes the net antigen-binding force of antibodies. Following primary antigenic challenge, the initially produced antibodies have low affinity that increases progressively over weeks or months by antigen driven B-cell selection process. The addition of protein denaturing agents disrupts the antigen-antibody link. This was found to have little effect on the high avidity antigen-antibody link but great effect on that of weak avidity antibodies. The measurement of avidity was determined by using the ratios of optical densities of the samples with and without the denaturing agent\(^14\).

Although the clinical ophthalmological diagnosis is still held to be the “gold standard” among all diagnostic efforts for active ocular toxoplasmosis, yet other causes as herpetic retinitis and metastatic endophthalmitis may closely mimic the signs of toxoplasmosis\(^15\). Also, in some instances, misdiagnosis may occur when the active retinal lesions are associated with haemorrhage and vitritis that hinder the fundus examination\(^16\). Thus, the delayed diagnosis of toxoplasmosis can lead to delayed anti-toxoplamic therapy, hence aggravation of the retinal lesions with
serious complications that may occur with even visual loss if the macula was affected.

Owing to the high risk of flaring up of intraocular inflammation and endophthalmitis, the detection of local specific antibodies in either vitreous or aqueous humour should be avoided. Instead, tear sampling is a safe, simple, non-invasive and practical technique for investigating the presence of local specific antibodies in active ocular toxoplasmosis. The present study aims at detection of anti-Toxoplasma IgA, IgM and IgG, as well as Toxo IgG avidity in the tear samples of patients with suspected ocular toxoplasmosis as a marker of early detection of human ocular toxoplasmosis.

SUBJECTS AND METHODS

Patients attending the Outpatient Clinic of Ophthalmology Department–Tanta University Hospital during the period from April 2005 to June 2006 were subjected to routine fundus examination and those suspected to have ocular lesions compatible with ocular toxoplasmosis were selected. The suspected cases were subjected to full history taking including: age, sex and any obstetric complications in women patients. All patients were further examined looking for extraocular signs of toxoplasmosis.

The selected cases presented retinal inflammatory lesions consistent with ocular toxoplasmosis and the diagnosis was made by fundus examination using direct and indirect ophthalmoscopy as well as fluorescein angiography using the criteria defined previously by Holland et al. As in order to consider active posterior uveitis induced by Toxoplasma gondii as present, the following clinical aspects were defined: scarred retinochoroiditis with well defined borders, suggestive signals of hyperthrophy and atrophy of the pigmentary epithelium together with satellite lesions showing inflammatory activity, different degrees of retinal vasculitis, and vitreal damage.

The patients were classified according to their clinical ocular history and the results of fundus examination into two groups:

Group (I): consisted of 14 patients with signs of active posterior uveitis presumably due to toxoplasmosis (APUPT) with or without old retinal scars at examination.

Group (II): consisted of 26 patients with chronic retinochoroidal lesion without active uveitis. They had old scars and without retinal inflammatory signs at the moment of sampling.

A third group consisted of 20 individuals who were chosen among healthy volunteers, without any ocular or systemic illness and considered as a control group (group III).

Tear samples were collected from each subject as well as serum samples and were investigated for anti-Toxoplasma IgA, IgM and IgG. The positive samples for anti-Toxoplasma IgG were further investigated for Toxo-IgG avidity.

Sample collection:

Tear samples: Tears were collected with a standardized Schirmer-plus® filter paper (3 cm length × 0.5 wide). The paper strip, folded at 0.5 cm from the end, was inserted into the lower conjunctival fornix laterally for 5 minutes. There was no stimulation of tear production. Before analysis, the filter paper strips, containing tears, were cut to the weight of 50 mg. Tears were extracted from weighed filter paper with 2 ml of PBS 0.01 M pH 7.2 under heavy shaking with touch mixer.

Blood samples: 5 ml of venous blood was collected under aseptic conditions from each investigated subject. Samples were transferred to plain tubes, allowed to clot and centrifuged for 5 minutes at 2000 rpm. Serum was separated, collected and stored at -20°C until analysis.

Estimation of anti-Toxoplasma IgA:

This was done by Toxo IgA ELISA kits (DiaMED Eurogen, Belgium.Cat.No.710021). The "IgA antibody capture" principle was used in this assay. All reaction steps were performed according to the manufacturer instructions. The enzymatic reaction was stopped by the addition of acid and the absorbance values at 450 nm were recorded. A standard curve was obtained by plotting each absorbance value versus the corresponding standard value. In order to obtain quantitative result for Toxoplasma IgA, the value of each patient sample, was compared with the established cut-off values. The Toxo IgA antibody concentration was presented in arbitrary A-units/ml. A result > 60 AU/ml was considered positive (clearly detectable Toxo IgA antibody).
Estimation of anti-Toxoplasma IgM: This was done by the VIDAS TOXO IgM (TXM, REF: 30 202, BioMerieux) which is an automated assay for the VIDAS system. The assay principle combines the enzyme immunoassay method with a final fluorescent detection (ELIFA). After IgM immunocapture, the anti-Toxoplasma IgM were specifically detected with alkaline phosphatase labeled immune complex. All reaction steps were performed by the VIDAS instrument, according to the manufacturer instructions. The results were analyzed automatically and the index \(i\) was calculated by the VIDAS instrument. Results were expressed on the result sheet as: index \(i \geq 0.65\) was considered positive.

Estimation of anti-Toxoplasma IgG: This was done by the VIDAS TOXO IgG (TXG, REF: 30 210, BioMerieux). The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELIFA). All reaction steps were performed by the VIDAS instrument, according to the manufacturer instructions. The results were analyzed and calculated automatically by the instrument using calibration curves. Results were expressed on the result sheet in IU/ml with a titer \(\geq 8\) was considered positive.

Estimation of Toxo-IgG avidity: This was done by the VIDAS® TOXO IgG Avidity (TXGA, REF: 30 222, BioMerieux). The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELIFA). All reaction steps were performed by the VIDAS instrument, according to the manufacturer instructions. The test used a dual strip comprising one reference strip and one test strip. The sample to be tested, after dilution, was dispensed into both sample wells of the dual strip: reference and test. Any anti-Toxoplasma IgG present in the sample formed complexes with the antigen coated to the solid phase. In the reference strip, non-specific antibodies were eliminated by washing, whereas specific antibodies remained coated to the solid phase. In the test strip, washing with the dissociating agent changed antigen-antibody links. Only antibodies with high avidity remained bound to the solid, whereas antibodies with low avidity were eliminated. Alkaline phosphatase labeled human anti-IgG antibody (conjugate) was then cycled in and out of the solid phase receptacle (SPR), and bound with any human IgG coated on the interior of the SPR. Unbound conjugate was removed by washing. The reaction medium was cycled in and out of the SPR several times. During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzed the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferylone), the fluorescence of which was measured at 450 nm. The intensity of the fluorescence was proportional to the concentration of antibodies present in the sample. The ratio between the quantity of high avidity antibodies (test strip) and the quantity of total antibodies (reference strip) provided an index which indicates antibody avidity in the tested sample. An avidity index \(< 0.200\) indicated low avidity IgG denoting recent infection within the last 4 months, whereas index \(\geq 0.300\) indicated high avidity IgG. Index range from \(\leq 0.200\) to \(< 0.300\) indicated borderline avidity.

Statistical analysis: Data were expressed as means ±SD and. Results were analysed statistically where \(P\) values < 0.01 were considered significant by using the Fisher's exact test and Mann-Waltney test. This was performed by SPSS software program version 13.

RESULTS

The results of anti-Toxoplasma immunoglobulin assay (including IgA, IgM, and IgG as well as IgG avidity) in paired tear and sera samples of 14 patients of group I with active posterior uveitis presumably due to toxoplasmosis (APUPT) were summarized in table (1). Anti-Toxoplasma IgM antibodies were not detected in any of tear or serum samples of all subjects. For tear samples, It was found that 9 out of 14 examined patients (64%) had positive slgA (72.22±2.77). In addition, 10 out of 14 patients (71%) had positive IgG values (98.9±86.8) in their tears, nine of them had low avidity IgG (0.156 ±0.03) with only one patient had high avidity IgG (0.354). The simultaneous presence of slgA and low avidity IgG in the tears of those 9 patients denoted recent active infection. The clinical data of those patients were presented in table (2) in which the range of age was 11-33 years with 6 females and 3 males. Patient number (6) in table (2) was a pregnant woman in the second trimester. The funduscopic picture and fluorescein angiogram of her left eye showed active macular toxoplasmosis on top of old lesions, while her right eye showed an
old macular toxoplasmic scar (Fig. 1). Regarding serum samples of patients of group I : there were 5 out of 14 patients (36%) with positive IgA (65.8±1.92) and 8 out of 14 patients (57%) with positive IgG (126.13±87.3). Two out of 14 serum samples (14%) had low avidity IgG (0.146±0.01). There was a significantly increased values of low avidity IgG in tears (P <0.01) as compared to that in serum samples.

In group II, table (3) showed that anti-Toxoplasma IgM was absent in all tear and serum samples. Regarding tear samples, sIgA (68.5±0.71) could be detected in 2 out of 26 patients (8%), whereas 19 out of 26 patients (73%) showed positive IgG values (34.68±18.5) and all IgG had high avidity (0.330±0.02). In serum samples, there were 3 out of 26 patients (12%) showed anti-Toxoplasma IgA (63.0±2.0). In addition, 21 out of 26 patients (81%) had positive IgG (46.90±26.7) that all had high avidity (0.320±0.01). There was no statistical significant difference between the values of IgA, IgG and IgG avidity in tears and serum samples.

Table (4) showed the immunoglobulin assay in 20 control subjects (Group III). No IgM could be detected in either tear or serum samples, whereas only one subject out of 20 controls (5%) had IgA in both tear and serum samples. There were 4 out of 20 subjects (20%) presenting IgG (21.0±12.89) in tear samples with 7 out of 20 subjects (35%) having IgG (34.29±16.9) in serum samples. All IgG had high avidity in both tear and serum samples (0.318±0.01 and 0.326±0.02 respectively), with no significant difference between the values of IgG and IgG avidity of tears and serum samples.
Fig. (1) : The findings of fundoscopy and fluorescein angiography examination of patient (6) in group (I) : The left eye (L) shows hazy media with vitreous turbidity (Vitritis), the red arrow points to an active retinochoroiditis that demonstrates late intense fluorescence. The right eye (R) shows large discoid punched out hyperpigmented macular scar, with two small neighbour ones. It demonstrates hypofluorescent centre with a hyperfluorescent borders of atrophic RPE. There is normal disc fluorescence.
Table (1): Immunoglobulin assay in tear and serum samples of patients of group I.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tear</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG avidity</td>
<td>IgG avidity</td>
<td></td>
</tr>
<tr>
<td>Ig</td>
<td>IgM</td>
<td>IgA</td>
</tr>
<tr>
<td>Positive/ tested samples</td>
<td>0/14</td>
<td>9/14</td>
</tr>
<tr>
<td>%</td>
<td>0%</td>
<td>64%</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>72.22 ±2.77</td>
<td>98.9 ±66.8</td>
</tr>
</tbody>
</table>

*P < 0.01 is significant
Positive values for: IgM: I ≥ 0.65, IgA > 60 AU/ml, IgG 8 ≥ IU/ml.
For IgG avidity: Low avidity = index < 0.200, high avidity = index ≥ 0.300

Table (2): Clinical data of 9 patients in group I with simultaneous detection of sIgA and low avidity IgG in tear film

<table>
<thead>
<tr>
<th>No of patients</th>
<th>Sex</th>
<th>Age</th>
<th>Involved eye</th>
<th>Visual acuity at time of sampling</th>
<th>Visual acuity at end of attack</th>
<th>Duration of uveitis at time of sampling</th>
<th>Extraocular symptoms &amp; associated conditions</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>♀</td>
<td>33</td>
<td>Left</td>
<td>CF at 2 meters</td>
<td>CF at 3 meters</td>
<td>1 month</td>
<td>Pregnant in the second trimester</td>
<td>Spiramycin only</td>
</tr>
<tr>
<td>9</td>
<td>♀</td>
<td>19</td>
<td>Right</td>
<td>CF at 2 meters</td>
<td>CF at 3 meters</td>
<td>3 months</td>
<td>-</td>
<td>Cort. +Sp.</td>
</tr>
</tbody>
</table>

CF = Counting fingers, Cort.: Corticosteroids, Sp.: Spiramycin

Table (3): Immunoglobulin assay in tear and serum samples of patients of group II.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tear</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG avidity</td>
<td>IgG avidity</td>
<td></td>
</tr>
<tr>
<td>Ig</td>
<td>IgM</td>
<td>IgA</td>
</tr>
<tr>
<td>Positive/ tested samples</td>
<td>0/26</td>
<td>2/26</td>
</tr>
<tr>
<td>%</td>
<td>0%</td>
<td>8%</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>68.5 ±0.71</td>
<td>34.08 ±18.4</td>
</tr>
</tbody>
</table>
Table (4): Immunoglobulin assay in tear and serum samples of control subjects (group III).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tear</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG avidity</td>
<td>IgG avidity</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Positive/ tested samples</td>
<td>0/20</td>
<td>1/20</td>
</tr>
<tr>
<td>%</td>
<td>0%</td>
<td>5%</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>-67 ±12.9</td>
<td>21.0 ±0.01</td>
</tr>
</tbody>
</table>

DISCUSSION

Toxoplasmosis is one of the most prevalent human infection in Egypt. Nearly one billion people worldwide are infected with Toxoplasma gondii, and ocular lesions occur in up to 25% of infected individuals.

Diagnosis of ocular toxoplasmosis is often based on typical clinical appearance. The presence of an active yellowish-white lesion with pigmented border and perivasculitis in the retinochoroid helps the diagnosis. Unfortunately, it is quite difficult to diagnose active ocular toxoplastic infections in conditions where severe vitritis hampered the fundal view. In active ocular toxoplasmosis, the serological diagnosis is almost not conclusive. As it has been demonstrated that, retinochoroiditis usually remains a local event, toxoplasmosis does not necessarily evoke a detectable systemic immune response.

In the present study, all tear and serum samples of group I with active retinochoroidal lesions showed no anti-Toxoplasma IgM antibodies. This came in agreement with the results of Garweg et al. and Villard et al. who showed that no IgM was reported in serum or locally in aqueous humor of patients with reactivated ocular toxoplastic lesions. This could be explained on the basis that active posterior uveitis presumably due to toxoplasmosis (APUPT) is seldom produced during a prime-infection; but always occurred in reactivation of old infections as evidenced by the presence of scarred lesions in the posterior pole of the eye in most of the cases. Thus, a secondary immune response was produced with IgG production and absence of IgM. The reactivation of the initial retinal lesion presumably results in rupture of quiescent parasitic cysts lying adjacent to pre-existing retinal scars and may secondarily involve the choroids leading to retinochoroiditis.

In group II with chronic retinochoroidal lesions, 2 out of 26 patients (8%) had positive sIgA in their tears, whereas in group III, only one out of 20 control subjects (5%) had sIgA in the tear film. The presence of anti-Toxoplasma sIgA in
stimulating specific sIgA production that triggered the immunologic memory of the high prevalence of anti-Toxoplasma sIgA in their tears. The relatively high prevalence of anti-Toxoplasma sIgA in tears of healthy subjects in the studies of Meek et al.(11) and Lynch et al.(18) in comparison with the present study may be explained by the high endemicity of the Toxoplasma parasite in the communities of their studies which enhanced the omnipresence of T.gondii in the environment that triggered the immunologic memory of the mucosal immune system continuously, thus stimulating specific sIgA production(26).

As regards the results of anti-Toxoplasma IgA the serum samples, it was found that 5 out of 14 patients with APUPT (36%) had positive IgA. This result came in agreement with that of Ronday et al.(27) who reported 16% of positive serum IgA cases in confirmed ocular toxoplasmosis patients. Moreover, Lynch et al.(18) found that all the examined APUPT patients in his study had positive reactions for anti-Toxoplasma serum IgA. This would assure the significance of serum IgA as a useful marker for active toxoplasmosis. Regarding the level of serum IgA in group II with chronic retinochoroiditis, there were positive serum IgA levels in 3 out of 26 patients (12%). This was contradicted with other studies(28,29,30) that described the absence of serum IgA antibodies in chronic toxoplasmosis. In the control group (gp.III), only one out of 20 subjects (5%) had positive serum IgA. The presence of anti-Toxoplasma IgA in sera of control healthy subjects had been also detected by Lynch et al. (18) where 12% of the examined control subjects had positive serum IgA, that may be produced as an amnestic immunological reaction.

The results of the present study concerning the detection of IgG in the patients of group I with APUPT showed that 71% and 57% of patients presented anti-Toxoplasma IgG in tear and serum samples respectively. There was insignificant low titers of IgG (98.9±86.77) in the tears as compared with that in the sera (126.13 ±87.3). In addition there were 9 out of 14 patients (64%) who had low avidity IgG as well as sIgA in their tears. This simultaneous presence of sIgA and low avidity IgG in the tears of those nine patients strongly confirmed the diagnosis of active ocular toxoplasmosis. Whereas, there was only 2 patients who had low avidity IgG in their sera. The discrepancy between IgG avidity in tears and serum coincided with the findings of other studies(31,32). They found that low avidity IgG was presented locally in aqueous humor not in the serum of patients with active ocular toxoplasmosis, eventually indicating a higher turnover of antibodies within the eye in consequence of the disease activity. In addition, Hedman(33) and Paul(34) suggested that IgG avidity indices for the serum are not helpful in diagnosing active ocular toxoplasmosis. Moreover, Lappalainen and Hedman(14) demonstrated that the role of IgG avidity measurement is merely to confirm the presence of a latent infection and to raise the suspicion of an ongoing reactivation.

Regarding IgG results of group II and the control subjects, it has been found that 73% and 81% of patients with chronic retinochroidal lesions showed high avidity IgG antibodies in tear and serum samples respectively. Meanwhile, in the control group (gp.III) 20% and 35% of subjects had high avidity IgG in their tears and sera respectively. The present results came in agreement with other studies that detected a high percentage of the volunteers having anti-Toxoplasma IgG antibodies in their serum, indicating a high prevalence of T. gondii. This was attributed to the cosmopolitan distribution of toxoplasmosis where most infected adults were symptomless with no visual impairment although they had positive specific IgG in their sera (18,35).

It is of particular interest to mention that one patient among the nine patients of group I who showed simultaneous presence of sIgA and low avidity IgG in their tears, was a pregnant woman in the second trimester and she had the episode one month before the time of sampling. This would support the work of Garweg et al.(18) and Kump et al.(36) who suggested that pregnancy may provoke recurrence of ocular toxoplasmosis. Their studies showed that pregnant women developed recurrences of ocular toxoplasmonic lesions that would be severe, treatment-resistant and could proceed to marked decreased visual acuity in spite of aggressive therapy.

The results of the present study showed the possible detection of local specific antibodies particularly sIgA in the tear film in a sufficient amount as the lacrimal gland is an effector organ of the common mucosal immune responses. Hence, the tear fluid can be considered as a possible candidate for investigating the presence of local anti-Toxoplasma antibodies because its collection is noninvasive, simple and easy as compared to the risky puncture of the eye to
collect other local ocular fluids as aqueous humor and vitreous. In conclusion, the present study suggested that the simultaneous detection of anti-Toxoplasma sIgA and low avidity IgG in tear samples could be considered as a useful marker for early diagnosis of active ocular toxoplasmosis that would help in early specific anti-Toxoplasma treatment to prevent serious complications.

REFERENCES


تهدف هذه الدراسة إلى تشخيص التوكسوبريلما النشطة في العيون بواسطة اكتشاف وجود
المضادات المضادة الموضعية المضادة للتوكسوبريلما (أ، م، ج) وكذلك شدة القدرة الارتباطية
للمضادات (ج) المانية الموجودة في الدموع.

تم عمل فحص لقاع العين للمرضى المترشدين على العيان الخارجية لقسم العيون
بالمستشفى الجامعي بطنطا في الفترة بين أبريل 2005 ويومنا 2006، ثم اختيار الحالات
المثبتة في إصابتها بمرض التوكسوبريلما في العيون، حيث تم تقديمهم إلى
المجموعة المختارة (1) وتشمل على 14 مريض يثبت في إصابتهم بالإتلايبوب الدخلي لعينية
العين النشط الناتج عن التوكسوبريلما. المجموعة (2) وتشمل على 26 مريض يثبت في
إصابةهم بالتهاب التوكسوبريلما المزمن للعيون والشمم. كما تم اختيار عشرين شخص لحص مس
من المتغيرات الإحصائية كمجموعة ثانية ضابطة (المجموعة 3). تم جمع عينات الدموع و
الأملاح من جميع الأشخاص في كل المجموعات. هذا وقد تم فحص جميع العينات لاختبار
وجود المستضادات المضادة للتوكسوبريلما (أ، م، ج) وكذلك شدة القدرة الارتباطية
للمضادات (ج) المانية.

أظهرت نتائج هذا البحث أنه في المجموعة (1) تم الكشف عن وجود المستضادات
المناعية المضادة للتوكسوبريلما (أ) وكذلك المستضادات المضادة الموضعية للتوكسوبريلما (ج)
المخفضة القدرة الارتباطية في عينات الدموع الخاصة بسعة أشخاص من عدد 14 شخص
(9%) مما يدل على وجود التهاب نشط في العين. في حين كانت نسبة وجود المستضادات
المناعية المضادة للتوكسوبريلما (أ، م، ج) في عينات أملاح المجموعة الأولي منخفضة. أما في
المجموعة (2) ووجد 8% من عينات الدموع المرضي تحتوي على المستضادات المناعية المضادة
التوكسوبريلما (أ) بينما كان 73% من المرضى لديهم المستضادات المناعية المضادة
التوكسوبريلما (ج) المرتفعة القدرة الارتباطية في عينات الدموع الخاصة بهم. بينما بلغت
نسبة المستضادات المناعية المضادة للتوكسوبريلما (أ، م، ج) في أملاح المرضي 12% و
81% على التوالي. أما في المجموعة الضابطة (3)، فقد وجد شخص واحد فقط محتوي عينات
الدموع والم yaln الخصى المضادة للتوكسوبريلما (أ، م، ج). بينما كانت
نسبة المستضادات المناعية المضادة للتوكسوبريلما (أ، م، ج) في كل من عينات الدموع والمسلام
20% و35% على التوالي حيث كانت شدة القدرة الارتباطية لمستضادات (ج) المناعية مرتفعة
في كل العينات. هذا ولم يتم الكشف عن وجود المستضادات المضادة للتوكسوبريلما
(م) في عينات الدموع والاملاح الخاصة بجميع الأشخاص في كل المجموعات.

أوضح نتائج هذا البحث أن تزامن وجود كل من المستضادات المناعية المضادة
التوكسوبريلما (أ) وكذلك المستضادات المضادة الموضعية للتوكسوبريلما (ج، المخفضة القدرة
الارتباطية في عينات الدموو مثل دالة لتفتيش المزمن للتوكسوبريلما النشط في العيون.
كما أوضح البيان أيضاً أمكنية استخدام الدموو في الكشف عن وجود المستضادات المناعية
الموضحة في العين حيث أن الحصول على الدموو وسيلة أمينة وبسيطة بالمفارقة بطريقة الحصول
على السائل المائي والسائل الزجاجي للعين.

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