Serological investigation of ocular toxoplasmosis

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Abstract

The limitations of serological assessment in toxoplasma infection of the eye are well recognised, but the predictive value of clinical examination is not defined. We undertook a prospective investigation into the role of clinical examination and of serological findings in cases of suspected toxoplasma infection of the eye by means of the dye test and multiple IgM assays. Seventy-four cases of retinal disease and 202 control patients were studied. Patients with retinal disease had a significantly higher incidence of toxoplasma seropositivity than the control group. This was because some patients with retinal disease had acquired the infection congenitally. Half the patients investigated for toxoplasmosis were seronegative. Possible explanations for these findings included misdiagnosis, clinical uncertainty, or, the use of serology testing in the confirmation of other diseases. An excess of IgM reactivity among the retinal disease group may indicate low level immunoglobulin-M production associated with an acute exacerbation of ocular toxoplasmosis. There is a need to consider invasive procedures in cases of ocular infection and for novel techniques to aid the diagnosis of toxoplasma retinochoroiditis.

Ocular toxoplasmosis is believed to be the most common infectious disease involving the retina.[1] The great majority of cases are thought to be associated with the late sequelae of exposure in utero to the parasite.[2] This opinion is largely based on epidemiological evidence. It has been estimated that less than 2% of patients with toxoplasmic lymphadenopathy have retinochoroiditis related to acquired infection.[3] In contrast, a prospective study of toxoplasmosis in pregnancy found that 46 of 210 (22%) congenitally infected infants suffered chorioretinitis.[4] These figures may underestimate the problem owing to referral bias noted during the study.

Population studies have shown that the prevalence of toxoplasma infection increased with age. Consequently, if toxoplasma retinochoroiditis was associated with post-natally acquired infection, the numbers of cases should rise with increasing age. However, toxoplasma chorioretinitis is most frequent in the second and third decade of life and rare after the age of 50 years. Further evidence is provided by the lack of serological findings indicating acute infection associated with toxoplasma ocular disease and the observation that most cases show the presence of old scars at the time of diagnosis, suggesting previous activity.[3]

A confirmed diagnosis of ocular toxoplasmosis indicates the need for extended ophthalmic supervision, with prompt institution of short-course specific therapy during episodes of acute exacerbation.[5] The toxic effects of the antiparasitic agents employed are well recognised. Consequently there is a need to establish a definite diagnosis of ocular toxoplasmosis as early as practicable.

Measurement of specific antibodies in the peripheral circulation has significant limitations when toxoplasmic retinochoroiditis is suspected. Immunoglobulin-M is rarely detected in association with ocular toxoplasmosis when standard assays are utilised. Although virtually all patients with the condition have detectable specific IgG, the relatively high prevalence of asymptomatic toxoplasmosis in the normal population markedly reduces the specificity of this finding.[6] Ocular tissues are rarely made available for histological examination, and analysis of aqueous humour, as carried out in other countries, is undertaken infrequently in the United Kingdom.[7] Therapeutic trials of antitoxoplasma drugs are difficult to assess, partly owing to the concurrent administration of systemic steroids.[5] In practice the diagnosis of ocular toxoplasmosis is most often based on clinical findings, and the presence of specific antibodies in serum is used as supportive evidence to a variable extent. However, the signs of ocular toxoplasmosis are diverse,[8] and the criteria for clinical diagnosis may vary from one ophthalmologist to another.

As a reference laboratory serving a number of specialist eye units we receive a large number of requests for the serological assessment of patients with retinal disease in whom toxoplasmosis is included in the differential diagnosis. We have therefore undertaken a prospective study to try to elucidate the relationship, if any, between clinical diagnosis and the results of laboratory tests for toxoplasma-specific antibodies.

Patients and methods

Patients were entered into the study on receipt of a serum sample with a request for toxoplasma investigation when the age and sex of the individual were stated and clinical signs of retinal disease were noted by the ophthalmologist. Only patients examined at centres known to refer samples for toxoplasma studies without prior testing or selection were included in the study. Centres practising selective referral were excluded. A control group was recruited from healthy blood donors in the same geographical region as the study group. Control samples were available only from individuals aged between 20 and 69 years. Consequently, patients with retinal disease with a recorded age beyond this range were excluded from the study.

Toxoplasma specific antibodies were measured by the dye test[9] applied to serial dilutions beginning with neat serum. The dye test is predominantly an assay of toxoplasma-

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specific immunoglobulin-G. A sample was defined as positive when found to contain ≥2 IU specific antibody. Immunoglobulin-M was measured by a double sandwich enzyme-linked immunosorbent assay (DS-ELISA) and an immunosorbent agglutination assay (ISAGA).[10] Statistical analysis was performed by means of the χ^2 test.

Results

A total of 111 patients with retinal disease were investigated during the course of the study. In 22 cases the age of the patient was not available, two patients were of unstated sex, seven were under 20 years old and six were aged 70 or more. These cases were excluded from further assessment. Of the remaining 74 patients comprising the study group 42 were males and 32 females, average age 36 years. The control group consisted of 202 individuals, 100 males and 102 females, average age 40 years. Toxoplasma serological findings are presented in Table 1. Of the 74 patients with retinal disease 40 (54%) had detectable toxoplasma – specific IgG compared with 45 of 202 controls (22%). Within the retinal disease group 12 patients had specific IgM detectable by ISAGA only, and one patient's serum was reactive by ISAGA and DS-ELISA. Two individuals in the control group were reactive by ISAGA alone and one by both ISAGA and DS-ELISA.

After correction for age and sex, and on the assumption of linear acquisition of antibody with increasing age, the calculated annual seroconversion rates were 1·5% and 0·6% for test and control groups respectively. The difference in seroconversion and seropositivity between the groups was statistically significant ($p<0·001$). Although DS-ELISA reactivity was comparable in the two groups, ISAGA reactivity was significantly more frequent among the retinal disease patients ($p<0·001$).

Table 1 Toxoplasma serological findings in patients with retinal disease and healthy individuals analysed by age and sex

			IgG		IgM	
Age (years)	*Patient group*	*Sex*	*Dye test negative*	*Dye test positive*	*DS-ELISA positive*	*ISAGA positive*
10–19	Retinal disease	M	5	5	0	2
		F	4	1	0	0
	Control	M	4	1	0	0
		F	14	0	0	0
20–29	Retinal disease	M	4	6	0	3
		F	2	4	0	0
	Control	M	21	1	1	1
		F	15	4	0	0
30–39	Retinal disease	M	4	1	0	1
		F	3	4	0	2
	Control	M	14	6	0	1
		F	17	2	0	0
40–49	Retinal disease	M	3	4	0	2
		F	2	6	0	2
	Control	M	13	7	0	0
		F	17	4	0	0
50–59	Retinal disease	M	3	3	0	0
		F	1	1	0	0
	Control	M	13	7	0	1
		F	14	5	0	0
60–69	Retinal disease	M	1	3	1	1
		F	2	2	0	0
	Control	M	8	5	0	0
		F	7	3	0	0
Total	Retinal disease		34	40	1	13
	Control		157	45	1	3

Discussion

Although patients referred from centres known to undertake on-site toxoplasma serological investigation were excluded from the evaluation, the study findings are susceptible to bias introduced by unrecognised primary screening. Serotesting in a non-reference laboratory with selective referral may result in a falsely high seropositivity rate among the retinal disease group. Any study of disease prevalence based on serological investigation will be influenced by the sensitivity and specificity of the assays employed. The dye test is the accepted reference assay for the measurement of toxoplasma specific antibody.[11] False positive reactions are rarely produced by this test, and, when performed on neat serum, the extreme sensitivity of the assay is widely recognised. False negative dye test results have not been recorded in immunocompetent individuals.

Performance characteristics of toxoplasma specific IgM assays are less well defined, reflecting the difficulties of obtaining reference samples. However, clinical experience of the two immunoglobulin-M tests used is extensive, and a comparative evaluation of these assays has been performed.[10] The retinal disease and control group were found to be of comparable age and sex distribution. It is unlikely that the differences observed between the groups of patients were associated with the influence of these factors.[12]

The reasons for requesting toxoplasma investigation in the group of patients with retinal disease were varied. Serotesting may be requested when the clinical findings are characteristic of ocular toxoplasmosis. In addition, when the signs and symptoms are discordant with toxoplasma infection, serology findings may be used to exclude this diagnosis. A third category of patients will comprise individuals where the clinical findings are suggestive but not definitive, and additional information derived from laboratory investigation is needed for the final diagnosis. Other than when used to exclude toxoplasmosis, negative serological findings must indicate misinterpretation of clinical findings or 'false negative' laboratory results. Specific IgG detectable by the dye test persists for many years following exposure to the parasite, whether acquired in utero or during the postnatal period.[11] Given the sensitivity of dye testing of neat serum, a negative reaction is unlikely to be associated with loss of detectable antibody with the passage of time following initial exposure. In addition, negative toxoplasma serology using multiple assays has not been recorded in cases of acute or chronic infection of the immunocompetent. Consequently the finding of non-reactivity in 47% of patients with retinal disease investigated for toxoplasmosis is of interest. Insufficient details of the original clinical opinion were available to distinguish between misdiagnosis, confirmation of non-toxoplasma aetiology, or clinical uncertainty. An American study has suggested that a relative overdiagnosis of ocular toxoplasmosis occurs when based on clinical findings.[13]

Future analysis of the relation between clinical

findings and the results of laboratory tests would be greatly assisted if ophthalmologists stated their diagnosis when requesting toxoplasma investigation. The signs and symptoms of ocular toxoplasmosis are varied, and the differential diagnosis is extensive.[8] Our findings indicate that further assessment of the predictive value of clinical examination in this condition is required. A minority of cases of retinal disease are confirmed by histological study, and the sensitivity and specificity of clinical opinion would be difficult to determine. However, the consistency and reproducibility of clinical diagnosis could be evaluated by presenting photographic slides of the retinal appearance in a number of different cases to a group of ophthalmologists and comparing the clinical interpretation of the data.

We have found significantly greater toxoplasma seropositivity among the retinal disease patients than the control group. The seropositivity and calculated seroconversion rate recorded for the control group is comparable to the findings in other regions of the United Kingdom.[12] It has been found that the duration of persistence of detectable toxoplasma specific IgM following acute infection depends on the sensitivity of the assay used and individual variation of the immune response.[10] On average, specific IgM is detected in our laboratory for eight months by DS-ELISA and 18 months by ISAGA. If the IgG seroconversion rate in a series of 202 patients is 0·6%, one would expect to find one individual reactive by DS-ELISA and three having specific IgM detectable by ISAGA at the time of investigation. Consequently, the number of patients in the control group found reactive by each IgM assay is compatible with the calculated rate of IgG seroconversion.

The single blood donor found to be reactive by both ISAGA and DS-ELISA was likely to have acquired toxoplasmosis within eight months of investigation. The two control group patients who were reactive by ISAGA only were probably infected between eight and 18 months previously. These infections probably passed unrecognised, as do most cases of acute toxoplasmosis.

The calculated seroconversion rates derived from the present study are based on the assumption that acquisition of toxoplasma antibody is linear throughout the age range of the population. It is been demonstrated that there is no marked variation in the seroconversion rate between different age groups in the postnatal period.[14] However, if a significant number of cases of congenital infection are included within a study population, the calculated overall seroconversion rate will exceed the actual numbers of acquired infections recorded each year. A calculated seroconversion rate is obtained by dividing the total numbers of patients with evidence of infection by the combined age of the group. Consequently a significant number of infections acquired in utero, as would be expected among a group of patients with retinal disease, will result in non-linear distribution of the seroconversions within a population.

The single patient with retinal disease found to be reactive by DS-ELISA is comparable with the findings in the control group. However, there was an excess of serum samples from patients with retinal disease reactive by ISAGA. It has been demonstrated that the sensitivity of ISAGA is significantly greater than that of DS-ELISA for the detection of toxoplasma specific IgM, whereas both assays are highly specific.[10] In consequence, ISAGA is the assay of choice for the investigation of neonatal and fetal samples.[15] A possible explanation for the increased numbers of ISAGA reactions noted could be low level IgM production during an acute exacerbation of ocular toxoplasmosis.

Previous studies have not detected toxoplasma specific IgM associated with acute exacerbation of ocular disease but have used relatively insensitive methods to measure immunoglobulin-M. Our findings suggest the level of IgM production associated with an acute exacerbation of retinal disease was too low to be measured by the less sensitive DS-ELISA method but was detected by the more sensitive ISAGA technique. If confirmed, this finding would be of considerable diagnostic value, and further investigations of this aspect are required. Serial investigation of toxoplasma serology in patients with ocular toxoplasmosis during episodes of acute exacerbation of retinal disease and quiescence would be valuable.

We have demonstrated some of the limitations in the diagnosis of ocular toxoplasmosis by clinical and serological investigation. The role of aqueous humour analysis in selected cases requires consideration, and there is a need for novel diagnostic measures for cases of suspected toxoplasma infection of the eye.

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