Epidemiol. Infect. (1999), **122**, 299–303. Printed in the United Kingdom © 1999 Cambridge University Press

The relationship between ocular toxoplasmosis and levels of specific toxoplasma antibodies

D. J. CHAPMAN[1]*, D. ASHBURN[1], S. A. OGSTON[2] AND D. O. HO-YEN[1]

[1] *Scottish Toxoplasma Reference Laboratory, Microbiology Department, Raigmore Hospital NHS Trust, Inverness IV2 3UJ*
[2] *Department of Epidemiology and Public Health, Medical School, Ninewells Hospital, Dundee*

(*Accepted 23 November 1998*)

SUMMARY

The relationship between ocular toxoplasmosis and levels of toxoplasma specific antibodies was examined in 195 patients. Using clinical information collected by questionnaires, patients were divided into: 97 with ocular toxoplasmosis (group 1) and 98 with ocular lesions not due to toxoplasma (group 2). The geometric mean of dye test titres ($\pm$S.D. natural log titre) in group 1 was 53·2 ($\pm$0·95) compared with 24·6 ($\pm$1·11) in group 2 ($P < 0{\cdot}001$). Young females tended to have more active lesions compared with young males ($P < 0{\cdot}05$). There was an age-dependent difference in dye test titres between the groups ($P < 0{\cdot}001$). Group 1 showed a decline in titre with age compared with an increase in group 2. Ocular toxoplasmosis was diagnosed most frequently among 21–30 year olds. More group 1 patients had dye test titres $\geqslant$ 65 iu/ml than group 2 ($P < 0{\cdot}05$). Dye test titres $\geqslant$ 65 iu/ml support a diagnosis of ocular toxoplasmosis whereas lower titres suggest other causes for eye lesions.

INTRODUCTION

The limitations of serological investigations in the diagnosis of ocular toxoplasmosis are well recognized [1]. Most cases of ocular toxoplasmosis are diagnosed clinically [2] and serological tests are used to confirm previous exposure to *Toxoplasma gondii* or to exclude this disease [3]. The diagnostic value of serological testing for toxoplasma-specific antibodies is controversial. It has been said that there is no relationship between levels of specific antibodies and the presence of active ocular toxoplasmosis [4, 5].

Ocular toxoplasmosis has long been believed to be due to congenital infection and has been attributed only rarely to acquired infection [6]. This was because many cases of ocular toxoplasmosis presented within the first 2–3 decades of life and the belief was that if acquired infections were the cause of ocular toxoplasmosis, incidence would increase with age [6]. Recent work in America and France has led to the revised view that ocular toxoplasmosis in acquired infection may not be as rare as thought previously [3]. Both these theories place young adults at the greatest risk, both through maternal infection and acquired infection due to lifestyle. It has also been suggested that puberty may have an influence on susceptibility to infection, especially in females [7]. The aim of this study was to evaluate the relationship between serological data and ocular toxoplasmosis.

* Author for correspondence.

MATERIALS AND METHODS

The Scottish Toxoplasma Reference Laboratory receives serum specimens (regardless of results of local laboratory toxoplasma serology) from patients resi-

https://doi.org/10.1017/S0950268899002071 Published online by Cambridge University Press

Table 1. *Comparison of mean ages and geometric means of dye test titres in patients with ocular (group 1) and non-ocular (group 2) toxoplasmosis*

	Group 1		Group 2	
Number	91		86	
Mean age, years, (±2 S.D.)	40 (±38)		45 (±34)	
Geometric mean DT titres (± 1 S.D.*)	53·2 (±0·95)		24·6 (±1·11)	
Gender (no.)	F (56)	M (35)	F (43)	M (43)
Mean age, years, (±2 S.D.)	43 (±36)	36 (±40)	44 (±34)	45 (±34)
Geometric mean DT titres (±1 S.D.*)	58·4 (±0·97)	45·8 (±0·90)	28·9 (±1·04)	21·0 (±1·17)

* Standard deviation of natural log dye test titre.

dent in Scotland and Northern Ireland with ocular lesions that could be due to toxoplasmosis. Questionnaires are sent to clinicians caring for patients in whom a dye test titre $\geqslant 2$ iu/ml is obtained. Information is requested as to the patient's age, gender, clinical symptoms and date of onset of disease, treatment and information as to maternal and patient's possible sources of infection. From this questionnaire two groups of patients are identified, those clinically diagnosed as having ocular toxoplasmosis (group 1) and those whose ocular lesions are thought not to be due to toxoplasmosis (group 2).

We compared the distribution of gender, age and dye test titres, both between and within the two groups. By the nature of the questionnaire, information on clinical symptoms was confined to patients with ocular toxoplasmosis (group 1). Therefore, analysis was only carried out to find the relationship between dye test titres and clinical symptoms of active or quiescent ocular lesions, in conjunction with the gender and age of group 1 patients. The dye test was performed with neat sera followed by doubling dilutions from 1/2 to 1/2048, equivalent to 2–4000 iu/ml [8]. Statistical methods used in the analysis were χ^2 estimations, a general linear model, correlation coefficient and the t test. All analyses on dye test titres were performed after natural log transformation. Dye test titres were expressed as geometric mean ± one standard deviation of natural log of dye test titres.

Specific IgM was measured in all sera using a sensitive in-house screening test [9]. Positive or equivocal results were confirmed using a less sensitive commercial test (Toxonostika ELISA IgM, Organon Teknika, Cambridge, UK); when results were not confirmed using this method sera were also tested using the more sensitive TOXO ISAGA-M (Bio-Merieux, Basingstoke, UK).

RESULTS

Of the 195 patients from whom questionnaires were returned, 97 were diagnosed clinically as having ocular toxoplasmosis (group 1) and 98 were considered clinically not to have ocular toxoplasmosis (group 2). Questionnaires were completed satisfactorily by 91/97 group 1 patients (93·8%). The gender and age of patients in group 2 were obtained from the original request forms, full information being obtained for 86/98 (87·7%) patients. In group 1, 56 were female and 35 male, giving a female : male ratio of 1·6 : 1. In contrast, in group 2, the numbers of females and males were equal (43 females and 43 males). The difference in gender ratio between groups was not significant ($P > 0{\cdot}05$).

Mean ages, gender distribution and geometric mean of dye test titres of group 1 and group 2 patients are given in Table 1. A general linear model was used to compare natural logs of increasing dye test titres and increasing ages between the groups. The age by group was significantly different ($P < 0{\cdot}001$), indicating that there was an age-dependent difference between those with and without ocular toxoplasmosis. This was evidenced by the higher dye test titres found in the younger age groups of group 1 ($r = -0{\cdot}18$) compared with group 2. In contrast, dye test titres in group 2 increased with age ($r = 0{\cdot}31$) (Fig. 1). The ages in the two groups were 1–80 years. Figure 2 shows that the age band in which ocular toxoplasmosis was diagnosed most frequently was 21–30 years. Significantly greater numbers of group 1 than group 2 patients were aged $\leqslant 30$ ($P < 0{\cdot}05$).

https://doi.org/10.1017/S0950268899002071 Published online by Cambridge University Press

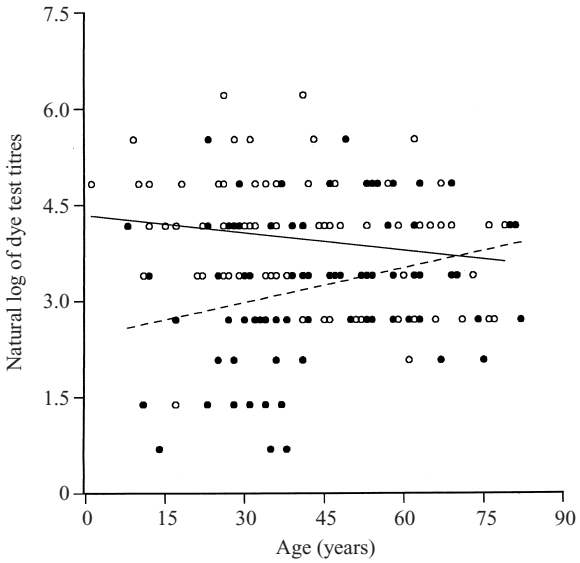

Fig. 1. Relationship between natural log dye test titre and increasing age in patients with (●) (group 1) or without (○) (group 2) ocular toxoplasmosis. Best fit lines were calculated using linear regression (group 1, solid line; group 2, broken line).

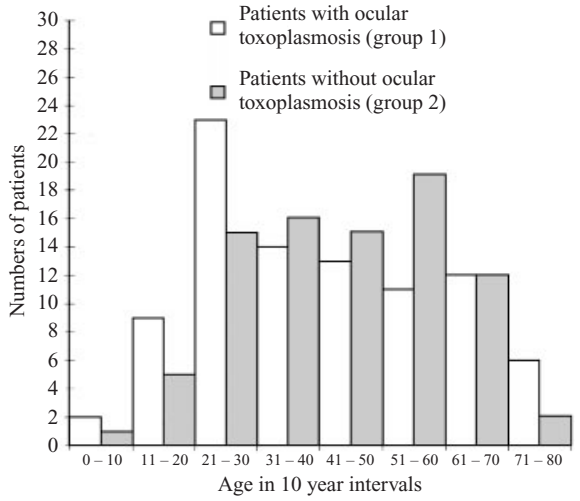

Fig. 2. Numbers of patients with and without ocular toxoplasmosis by age.

Dye test titres were 2–500 iu/ml in group 1 and 2–250 iu/ml in group 2. The geometric mean (±1 S.D. natural log of dye test titres) in group 1 was 53·2 (±0·95) and in group 2, 24·6±(1·11) iu/ml. Though the mean dye test titres in the two groups were separated by only one dilution, absolute values of patient numbers and dye test titres indicated that the highest frequency of observations in group 1 was 65 iu/ml and in group 2, 30 iu/ml (Fig. 3). To determine a dye test titre threshold which might be of value in differentiating between ocular and non-ocular

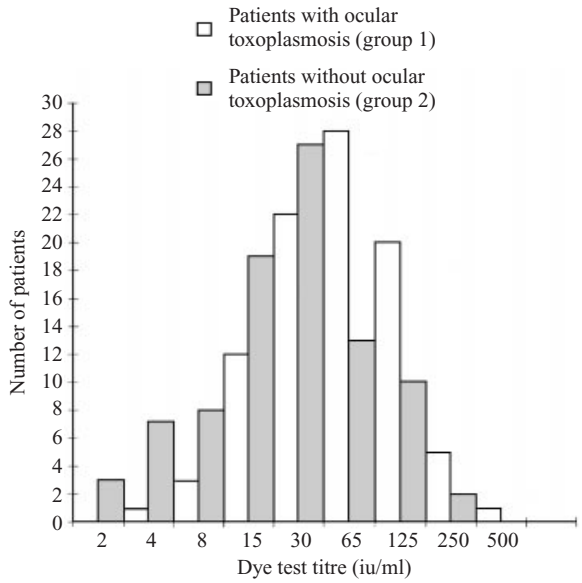

Fig. 3. Dye test titres in patients with and without ocular toxoplasmosis.

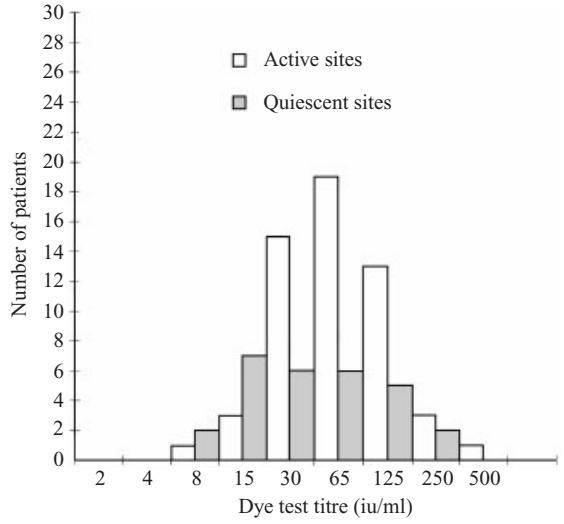

Fig. 4. Dye test titres in patients with active and quiescent sites.

toxoplasmosis, the numbers of patients with dye test titres ⩽ 30 iu/ml were compared with those with titres ⩾ 65 iu/ml. Group 1 contained significantly more patients with a dye test titre ⩾ 65 iu/ml than group 2 ($P < 0{\cdot}001$).

Within group 1, 53/91 (58·2%) patients had active or active and quiescent lesions compared with 27/91 (29·7%) with quiescent lesions only. No information as to the types of lesion was given for 11/91 (12·1%). Although more patients with active (with or without quiescent lesions) than quiescent lesions only had dye test titres ⩾ 65 iu/ml (Fig. 4), the difference between titres in each of these sub-groups was not significant

https://doi.org/10.1017/S0950268899002071 Published online by Cambridge University Press

($P = 0{\cdot}092$). Confirmed IgM positive results were obtained in five group 1 patients with active lesions. One patient with a quiescent lesion was also IgM positive. This may have been an acquired infection which was wrongly classified as being ocular toxoplasmosis. None of the group 2 patients was IgM positive.

DISCUSSION

In ocular toxoplasmosis, it has been believed that the presence of active lesions and the level of dye test titre bear no correlation [4]. Our work shows that although the majority of the dye test titres were within the normal range of 2–125 iu/ml, a significant number of patients, whose eye disease was classified clinically as ocular toxoplasmosis, had dye test titres in the upper normal range ($P < 0{\cdot}001$). This was in contrast to those patients whose ocular disease was not due to toxoplasmosis where dye test titres fell in the lower normal ranges ($P < 0{\cdot}001$). The clinical decision to request serology in cases of ocular toxoplasmosis is selective, based on individual practice, clinical findings and the patient's age, thus resulting in possible selection bias. Samples were received from many different sources, encompassing a variety of clinical practices. Therefore we believe that our results are a fair representation of the population with suspected ocular toxoplasmosis. As a Reference Laboratory, we encourage all of our users to refer samples from all patients with suspected ocular toxoplasmosis, as antibody levels may be low.

Ocular toxoplasmosis has been considered to be a sequel of congenital toxoplasmosis [6]. This is mainly because the majority of cases of ocular toxoplasmosis first present in the first 2–3 decades of life and rarely above 50 years of age which might be expected in acquired toxoplasmosis [6]. In our study, we demonstrated falling dye test titres with increasing age in patients with ocular toxoplasmosis. The opposite was true for patients with ocular lesions of other aetiology. Thus, whereas the latter demonstrate a typical acquisition of antibody with age [10], the former is more consistent with congenital infection. Production of toxoplasma IgM has been associated with exacerbation of ocular toxoplasmosis. Specific IgM positive results were recorded in 13/74 cases of retinal disease [1]. Our results confirm that IgM may be produced in a small proportion of cases of ocular toxoplasmosis, but its detection may be less useful than demonstration of dye test titres $\geqslant 65$ iu/ml.

There is good evidence to suggest that over 90% of cases of ocular toxoplasmosis are due to congenital infection [11]. Thus, it might be assumed that the incidence of ocular toxoplasmosis would be similar between the sexes. The distribution of gender within each group was different (in group 1, F:M = 1·6:1 whereas in group 2, F:M = 1:1), but not significant. However, the number of females below 30 years of age with active lesions was found to be almost 2·5 times that of males ($P < 0{\cdot}05$) and this may indicate an increased susceptibility of females to developing ocular toxoplasmosis. Alternatively, the female foetus may be more susceptible to congenital infection. Further studies are needed to examine this possibility.

Although most workers believe ocular toxoplasmosis to be a late sequel of congenital toxoplasmosis, workers in America and France have found that ocular toxoplasmosis due to an acute acquired infection may not be rare [3]. Work in Brazil has also suggested that host factors like puberty may be correlated with the onset of ocular toxoplasmosis. A study found a higher frequency of retinochoroiditis in young girls aged 13–16 years than in boys of the same age [7]. Although still within normal limits, we found a trend towards those patients who had an active form of the disease having a raised dye test titre. This, together with the finding that more young females tend to have active lesions than their male counterparts ($P < 0{\cdot}05$), may indicate that young females are at greater risk of severe ocular toxoplasmosis than young males. Differences in the incidence of ocular toxoplasmosis are associated with ethnic origin of patients [12]. This information was not requested in our questionnaire. However, as the majority of patients (97·4%) in our survey had British-sounding surnames, it is unlikely that this influenced the validity of our results.

The differential diagnosis of retinochoroiditis includes several infections as well as non-infectious causes [5] and because of similarities in the lesions, may result in an over-diagnosis of ocular toxoplasmosis. Although the absence of antibodies against toxoplasma exclude it as a cause, their presence does not confirm it. When higher antibody titres have been associated with ocular toxoplasmosis, there was correlation between increasing age and increasing titres both in the study and control groups [5]. This is quite different from our study where there was a positive association between titres and age in the group 2 but a negative association in group 1. Our results suggest that ocular lesions in the presence of

https://doi.org/10.1017/S0950268899002071 Published online by Cambridge University Press

dye test titres $\geqslant$ 65 iu/ml are indicative of ocular toxoplasmosis. Lower titres, while not excluding the possibility of ocular toxoplasmosis, may suggest that ocular lesions are due to other causes.

We have demonstrated significant differences in the relationship between dye test titres and age in patients with clinically diagnosed ocular toxoplasmosis and those with ocular lesions not due to toxoplasmosis. The highest incidence of ocular toxoplasmosis is in age group 21–30 years, suggesting the disease is a late manifestation of congenital infection. We believe that serological tests are an important contribution to diagnosis of ocular toxoplasmosis. Dye test titres $\geqslant$ 65 iu/ml are suggestive of ocular toxoplasmosis; lower titres indicate that alternative causes of lesions should be sought.

ACKNOWLEDGEMENTS

We would like to thank the clinicians and the laboratories who use the Scottish Toxoplasma Reference Laboratory, and who were good enough to complete our questionnaires. We are grateful to Ms Gillian Sweeney and Ms Debbie Gilham for secretarial assistance and to Dr Roger Evans for his help with the manuscript.

REFERENCES

1. Holliman RE, Stevens PJ, Duffy KT, Johnson JD. Serological investigations of ocular toxoplasmosis. Br J Ophthalmol 1991; **75**: 353–5.
2. Bordnand J, de Gottrau P. Uveitis: is ocular toxoplasmosis only a clinical diagnosis? Ophthalmologica 1997; **211**: 87–9.
3. Montoya JG, Remington JS. Toxoplasmic chorioretinitis in the setting of acute acquired toxoplasmosis. Clin Infect Dis 1996; **23**: 277–82.
4. Rothova A, van Knapen F, Baarsma GS, Kruit PJ, Loewer-Sieger DH, Kijlstra A. Serology in ocular toxoplasmosis. Br J Ophthalmol 1986; **70**: 615–22.
5. Swanson MW. Association of antibody titre and chorioretinal scarring in toxoplasma retinochoroiditis. J Am Optom 1989; **60**: 735–40.
6. Perkins ES. Ocular toxoplasmosis. Br J Ophthalmol 1973; **57**: 2–17.
7. Gomez Marin JE, Pinon JM, Bonhomme A, Guenounou M. Does human toxoplasmosis involve an imbalance in T1/T2 cytokines? Med Hypotheses 1997; **48**: 161–9.
8. Williams KAB, Scott JM, MacFarlane DE, Williamson JMW, Elias-Jones TF, Williams H. Congenital toxoplasmosis. A prospective survey in the west of Scotland. J Infect 1981; **3**: 219–29.
9. Joss AWL, Skinner LJ, Moir IL, Chatterton JMW, Williams H, Ho-Yen DO. Biotin-labelled antigen screening test for toxoplasma IgM antibody. J Clin Pathol 1989; **42**: 206–9.
10. Desmont G. Some remarks on the immunopathology of toxoplasmic uveitis. Mod Probl Ophthal 1976; **16**: 228–32.
11. Kihlstra A, Breebaart AC, Baarsma GS, et al. Aqueous chamber taps in toxoplasmic chorioretinitis. Doc Ophthalmol 1986; **64**: 53–8.
12. Gilbert RE, Stanford MR, Jackson H, Holliman RE, Sanders MD. Incidence of acute symptomatic toxoplasmosis retinochoroiditis in south London according to country of birth. BMJ 1995; **310**: 1037–40.

https://doi.org/10.1017/S0950268899002071 Published online by Cambridge University Press