A retrospective study of ocular toxocariasis in Japan: correlation with antibody prevalence and ophthalmological findings of patients with uveitis

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Abstract

To classify the clinical characteristics of ocular toxocariasis in Japan, the prevalence of antibodies to *Toxocara* antigens was examined in patients with uveitis of unknown aetiology. From 1982 to 1993, serum specimens of 383 cases and intraocular fluid samples of 22 cases were serologically screened for *Toxocara* infection with five immunodiagnostic tests. Fifty-five sera and 11 intravitreous fluid samples were estimated to have significantly high antibody levels against larval excretory–secretory (ES) antigens of *T. canis*. Eight cases were positive in both serum and vitreous fluid, and three were positive only in the vitreous fluid. Among the 58 antibody positive samples, 20 cases were omitted due to a lack of detailed description of ocular findings. The remaining 38 cases are described in this study. Of these 38 cases, 34 (89%) were older than 20 years of age. Ocular lesions were located in the posterior fundus in 11 cases, in the peripheral fundus in 18 cases, and in both areas in seven cases. Of the eight cases in which papillary oedema or redness was observed, chorioretinal lesions were also present in seven of them. Tractional retinal detachment was present in five cases. These observations suggest that ocular toxocariasis in Japan has a different clinical profile compared with those in the other countries, and indicate a need for revised classification of ocular toxocariasis.

Introduction

Toxocariasis is a zoonotic parasite infection caused by the larval stage of genus *Toxocara*, *T. canis*, or *T. cati*. Transmission of *Toxocara* to humans primarily results from the ingestion of food or soil contaminated with embryonated eggs of *Toxocara*. Once the eggs are ingested by humans, hatched larvae are able to invade the viscera, the central nervous system, and the skeletal muscle. The larvae are also able to migrate into the human eye, resulting in an irreversible vision defect due to the inflammatory response elicited by the larva. This is known as ocular toxocariasis. Accurate diagnosis of

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ocular toxocariasis depends on pathological detection of the larva in the affected eye (Wilder, 1950). Until now, only one confirmed case of ocular toxocariasis had been reported in Japan (Yoshioka, 1966). It is generally accepted that the larval excretory–secretory (ES) antigen is more specific for *T. canis* infection than other *T. canis*-derived antigens such as adult or larval extracts. Many researchers, therefore, consider that retinal lesions of unknown origin with high antibody levels of anti-*Toxocara* larval ES antibodies in serum or intraocular fluid may be ocular toxocariasis. In the last two decades, since ocular toxocariasis has become known to ophthalmologists, case reports of serologically confirmed toxocariasis have considerably increased in Japan. Unfortunately, however, almost all previous reports from Japan used the somatic antigen derived from adult worms or larvae for the serological diagnosis (Kuriyama *et al*., 1989; Seki *et al*., 1990; Tada, 1991; Fujii *et al*., 1993; Hijikata *et al*., 1995), although these antigens strongly cross-reacted with other helminth antigens. Moreover, serologically confirmed cases in Japan were diagnosed on the basis of the results obtained from only one serological test. In addition, few reports deal with statistical analysis of ophthalmological typing for ocular toxocariasis (Gillespie *et al*., 1993). The present study investigated the ophthalmological findings and the prevalence of anti-*Toxocara* larval ES antibody levels in patients diagnosed with uveitis of unidentified aetiology.

Materials and methods

From 1982 to 1993, serum specimens of 383 cases, 382 Japanese and a Korean, with uveitis of unidentified aetiology were referred to the Department of Parasitology, Kanazawa University School of Medicine for detection of the anti-*Toxocara* antibody. These sera were recieved from various prefectures in Japan. Nineteen samples of vitreous or aqueous fluid were obtained during therapeutic vitrectomy. As a control, 17 sera from aetiologically apparent and non-toxocariasis patients who had retinal lesions were also tested. These included patients with diabetes mellitus, rheumatoid arthritis, sarcoidosis, and Behçet disease. Three diabetic patients with retinal lesions were generously referred by Dr N. Morishima, Department of Ophthalmology, Tokyo Medical and Dental University. Using an enzyme-linked immunosorbent assay (ELISA), antibody levels were tested in serum samples obtained from 1737 individuals during routine medical examinations at several health centres in the Ishikawa Prefecture during the same period.

In this study, five immunodiagnostic tests were performed: ELISA, double gel diffusion test (DGDT), counter electrophoresis (CEP), immunoblot assay (IBA), and indirect fluorescent antibody test (IFA). Embryonated eggs of *T. canis* in JB-4 plastic embedded sections were used as the antigen for IFA. ES antigen from second-stage larvae of *T. canis* was used for the other tests. For serodiagnosis, first, antibody titres were measured in each sample by ELISA, then the other four tests were conducted if the ELISA was positive. To minimize the effects of ELISA plate-to-plate variation in results, the optical density of samples was converted to logarithms, and the values were compared to the ratio of the normal control samples (Kondo *et al*., 1998). The ELISA antibody was considered positive when the ratio was 2.1:1 or more. DGDT was performed as follows: 30 μl of unconcentrated serum and 8 μl of ES antigen (1 mg ml^{-1}) were placed in 0.9% agarose gel and incubated for 38 h in a moist chamber. Precipitin bands were visualized with Amido Black 10B. CEP was conducted as follows: the ES antigen was run in 0.9% agarose gel at 6 mA cm^{-1} constant current for 45 min, then sample serum was added. Precipitin bands were stained with Amido Black 10B. For IBA, polyacrylamide gel electrophoresis and a protein blot sheet were performed using the outlined method by Akao *et al*. (1989).

Ocular manifestations were classified on the basis of the location (posterior or peripheral) of the chorioretinal lesions and the presence or absence of an optic neuritis-like disc appearance. The appearance of the lesions (mass or exudative type) was neglected, because our longitudinal observation of two cases in the present study revealed that mass lesions sometimes develop on the sites of exudative lesions. These findings were also described by Yuasa (1994). Thus, we concluded that different findings in appearances do not have implications for a different nature of the lesions. Furthermore, the presence or absence of proliferation on the posterior hyaloid membrane was also omitted for the analysis because this change often develops after the prolonged presence of chorioretinal lesions. Fisher's exact probability test was used to check for independence of a contingency table between positive and negative results.

Results

Antibody prevalence

Table 1 shows the ELISA results for each group. The patients with uveitis of unknown aetiology had a significantly higher prevalence of anti-*Toxocara* antibody levels than the other two groups. All sera taken from patients with uveitis of an apparent aetiology were negative for the ES antigen. These results indicated that *Toxocara*-induced uveitis is almost certainly involved in uveitis of unknown aetiology.

Table 1. Antibody prevalence of patients with uveitis and normal individuals.

Classification of patient	No. examined	No. positive (%)
Uveitis of unknown aetiology	383	55 (14.3)
Uveitis of apparent aetiology*	17	0 (0.0)
Normal individuals	1737	26 (1.5)

*These include diabetes mellitus, rheumatoid arthritis, sarcoidosis and Behçet disease.

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Table 2. Antibody prevalence in the vitreous fluid of patients with uveitis of unknown aetiology and diabetes mellitus.

Classification of patient	No. examined	No. positive (%)
Uveitis of unknown aetiology	22	11 (50.0)
Uveitis w/diabetes mellitus	3	0 (0.0)

Table 3. Antibody prevalence in the serum and vitreous fluid of patients with uveitis.

		Antibody in serum	
		+	−
Antibody in vitreous fluid	+	8	3
	−	0	11

We examined 22 samples of vitreous fluid taken from patients with unknown aetiology. Fifty percent of the samples were positive, but all three diabetic patients with uveitis were negative (table 2). Eight patients had positive results in both serum and vitreous fluid, and three cases were positive only for vitreous fluid (table 3). Among the positive samples determined by ELISA, we further performed four immunological tests (table 4). The results of IFA correlated with those of ELISA; however, 23 of the ELISA-negative samples ($n=328$) were estimated to be positive. In contrast, none of the ELISA-negative sera was considered positive by DGDT, CEP, or IBA. These results indicated that the specificity of IFA is lower that the other tests. Because 20 of 58 cases were omitted due to a lack of detailed description of the ocular findings, the remaining 38 cases were described. In addition, three cases whose intravitreous fluid was positive for the *Toxocara* antibody were added to this study.

Clinical appearance of serologically diagnosed patients

A total of 38 cases (male, 27 cases; female, 11 cases) were diagnosed with ocular toxocariasis in this study. Unlike previous reports that stressed a predominance of ocular toxocariasis in children (Biglan *et al*., 1979; Searl *et al*., 1981; Brown & Tasman, 1981; Molk, 1982; Sharkey & Mckay, 1993; Del Castillo *et al*., 1995), 89% of the present cases (34 of 38 patients) were 20 years of age or older (table 5). All cases had uniocular involvement (right eye, 17 cases; left eye, 21 cases). A history of close contact with dogs and/or cats was noted in 23 cases (61%). Ten cases (26%) had a habit of eating raw meat and bovine or chicken liver. Twenty-six (68%) complained of visual loss or blurred vision, 16 (42%) of flying flies, and two (5%) of visual field defect (multiple complaints were repetitively counted).

Ophthalmological findings

As for the ocular findings, chorioretinal lesions, vitreous opacity, disc oedema or redness, and retinal vascular sheathing were noted in 36, 25, eight, and four cases, respectively (multiple findings were repetitively counted). Signs of anterior chamber inflammation (cell, aqueous flare, keratic precipitate, posterior synechia, etc.) appeared in eight cases. Chorioretinal lesions were classified into the three types. Namely, the posterior type has lesions around the papilla and/or within the vascular arcade (posterior pole), but not in the peripheral area. The peripheral type has lesions only outside the posterior polar area. The mixed type has lesions both in the posterior polar area and in the peripheral area. A total of 36 cases had chorioretinal lesions that included 11 cases of the posterior type, 18 of the peripheral type, and seven of the mixed type (table 6). Papillary oedema or redness was observed in eight cases. In these cases, seven cases had chorioretinal lesions. Among them, three were the posterior type, two were the peripheral type, and two were the mixed type.

Vitreous opacity was observed in 25 cases. Of the 25 cases, 23 were associated with chorioretinal lesions. Vitreous opacity was noted in six of 11 cases of the posterior type, 14 of 18 of the peripheral type, and three of seven of the mixed type. In the cases with vitreous opacity and chorioretinal lesion, vitreous opacity was always found in the vicinity of the chorioretinal lesion. In addition, 12 cases showed vitreoretinal traction to the chorioretinal lesion, an increase in the intravitreal fibrin,

Table 5. Distribution of sex and age of 38 cases of ocular toxocariasis.

Age	Male	Female	Total
0–9	0	1	1
10–19	2	1	3
20–29	1	2	3
30–39	13	4	17
40–49	8	3	11
50–59	2	0	2
60–69	1	0	1
Total	27	11	38

Table 4. Results of four immunodiagnostic tests in ELISA-positive patients.

	DGDT		CEP		IBA		IFA	
	+	−	+	−	+	−	+	−
Positive for ELISA (n=55)	53	2	26	19	46	9	55	0

DGDT, double gel diffusion test; CEP, counter electrophoresis; IBA, immunoblot assay; IFA, indirect fluorescent antibody test.

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Table 6. Ophthalmological findings of 38 cases of ocular toxocariasis.

	Location of the chorioretinal lesions				
Optic neuritis	Posterior	Peripheral	Mixed	None	Total
+	3	2	2	1	8
–	8	16	5	1	30
Total	11	18	7	2	38

or an invasion of neovascular structures into the chorioretinal lesion, resulting in retinal detachment in five cases. In one case, a white mass moved from the temporal side of the optic disc to the macula during a 3-month follow-up study, though a nematode was not identified.

Discussion

In Japan, Fujii *et al*. (1993) reported that 25 of 110 cases of uveitis showed a high anti-*Toxocara* antibody in serum. Hijikata *et al*. (1995) also reviewed 23 cases of ocular toxocariasis that were diagnosed on the basis of the ophthalmological findings and high antibody levels in serum. Unfortunately, the antigen used in these studies was not the ES product but somatic extracts of adults or larvae of *T. canis*. It is well-established that these somatic antigens highly cross-react with unrelated parasite antigens. In this study, larval ES products were used as the antigens for all immunological tests except IFA.

Among the patients with uveitis of unknown aetiology, 14% of them were positive for *Toxocara* antibody. Fujii *et al*. (1993) reported a high prevalence (23%) of antibody levels in patients with uveitis. A high prevalence (15%) was also demonstrated in the patients with uveitis of known aetiology. In the present study, in contrast, none of the samples from patients with uveitis of apparent aetiology, such as diabetes or Behçet disease, were positive for the *Toxocara* larval ES antigen. These results indicate that the larval ES antigen is more appropriate than somatic antigen derived from *T. canis* larvae.

In 1971, Wilkinson & Welch (1971) proposed a classification of ocular toxocariasis as follows: 1) endophthalmitis, 2) posterior pole granuloma, and 3) peripheral inflammatory mass. In the present study, however, 12 of 18 cases of the peripheral type had vitreous opacity, and therefore they did not clearly fit either type 2 or type 3 of Wilkinson–Welch's classification. Shields (1984) classified his cases into nine groups: 1) posterior retinochoroiditis, 2) peripheral retinochoroiditis, 3) optic papillitis, 4) endophthalmitis, 5) motile chorioretinal nematode, 6) diffuse unilateral subacute neuroretinitis, 7) keratitis, 8) conjunctivitis, 9) lens involvement. None of the 38 cases in the present study belonged to Shields's types 4 to 9, and, furthermore, most of our cases overlapped with multiple types of Shields' classification. Therefore, it is not practical to subdivide cases in Japan using Shields' classification.

Characteristic findings of ocular toxocariasis were endophthalmitis and posterior pole granuloma (Biglan *et al*., 1979; Searl *et al*., 1981; Kielar, 1983), or retinal granuloma (Gillespie *et al*., 1993). In Japan, however, over 70 to 80% of the serologically diagnosed cases had peripheral foci (Tada, 1991; Hijikata *et al*., 1995) and endophthalmitis is very rarely found (Yuasa, 1994), suggesting that ocular toxocariasis has a different clinical profile in Japan. In this observation, optic neuritis was one of the characteristic patterns of *Toxocara*-positive patients. Although this lesion was not included in Wilkinson–Welch's classification, *Toxocara* infection appears to be caused by optic neuritis (Brown & Tasman, 1981; Molk, 1982).

In conclusion, we propose practical criteria for the diagnosis and classification of ocular toxocariasis, at least in Japan. Firstly, ocular toxocariasis is presumed when serological tests using *Toxocara* larval ES antigen are positive either in the serum or intraocular fluid. Secondly, the classification is based only on the location (posterior or peripheral) of the primary chorioretinal lesion(s) regardless of their appearance (mass or exudative type) and associated with vitreoretinal traction signs. Disc appearance is affixed when the disc is oedematous or reddish.

Toxocariasis has been considered to be a disease in children who tend to have close contact with puppies and kittens or play in park sandpits which are frequently contaminated by *T. canis* eggs (Brown, 1970; Molk, 1983; Barriga, 1988). At least in Japan, however, adult patients were not a minority group, but rather comprised 89% of all cases in this study. Furthermore, several reports from Japan that described the incidence of toxocariasis in adults are consistent with our observation (Tada, 1991; Hijikata *et al*., 1995). Recent reports also revealed that *Toxocara* infection occurs after eating raw snails, meat, or liver (Ito *et al*., 1986; Nagakura *et al*., 1989; Romeu *et al*., 1991). Thus, the disparities between the age of onset and causative route can be attributed to this different classification.

Epidemiological studies using ELISA for *Toxocara* indicate that the positive rate in the general population of different countries varies from 0.7 to 14.6% (Barriga, 1988; Parker & Shaver, 1996), which indicates that latent infections are common. When uveitis or optic neuritis with heavy vitreous opacity occurs in an adult without apparent causes, toxocariasis must be included in the diagnostic table and serological tests should be performed.

References

Akao, N., Kondo, K. & Takakura, Y. (1989) Immunoblot analysis of human gnathostomiasis. *Annals of Tropical Medicine and Parasitology* **83,** 635–637.

Barriga, O.O. (1988) A critical look at the importance, prevalence and control of toxocariasis and the possibi-

https://doi.org/10.1017/S0022149X00700721 Published online by Cambridge University Press

lities of immunological control. *Veterinary Parasitology* **29,** 195–234.

Biglan, A.W., Glickman, L.T. & Lobes, L.J. (1979) Serum and vitreous *Toxocara* antibody in nematode endophthalmitis. *American Journal of Ophthalmology* **88,** 898–901.

Brown, D.H. (1970) Ocular *Toxocara canis*. II. Clinical review. *Pediatric Ophthalmology* **7,** 182–191.

Brown, G.C. & Tasman, W.S. (1981) Retinal arterial obstruction in association with presumed *Toxocara canis* neuroretinitis. *Annals of Ophthalmology* **13,** 1385–1387.

Delcastillo, J., Herreros, G., Guillen, J.L., Fenoy, S., Banares, A. & Garcia, J. (1995) Bilateral ocular toxocariasis demonstrated by aqueous humor enzyme-linked immunosorbent assay. *American Journal of Ophthalmology* **119,** 514–516.

Fujii, S., Tada, R., Nakagawa, Y., Jikuhara, Y., Fujioka, S., Haruta, Y. & Yuasa, T. (1993) Incidence of anti-*Toxocara* antibody in uveitis [in Japanese]. *Rin-Gan [Clinical Ophthalmology]* **47,** 173–176.

Gillespie, S.H., Dinning, W.J. & Crowcrft, N.S. (1993) The spectrum of ocular toxocariasis. *Eye* **7,** 415–418.

Hijikata, S., Fujita, K., Seki, F., Usui, M. & Tsuji, M. (1995) Review of twenty-three cases of ocular toxocariasis. *Journal of Clinical Ophthalmology* **49,** 1211–1214.

Ito, K., Sakai, K., Okajima, T., Ouchi, K., Funakoshi, A., Nishimura, J., Ibashi, H. & Tsuji, M. (1986) Three cases of visceral larva migrans due to ingestion of raw chicken or cow liver. *Journal of the Japanese Society of Internal Medicine* **75,** 759–766.

Kielar, R.A. (1983) *Toxocara canis* endophthalmitis with low ELISA titer. *Annals of Ophthalmology* **15,** 447–449.

Kondo, K., Akao, N., Ohyama, T. & Okazawa, T. (1998) Sero-epidemiological investigation of toxocariasis in Asian area. *Proceedings of the Fifth Asian-Pacific Congress for Parasitic Zoonoses*, 65–70.

Kuriyama, K., Ohna, S. & Tsuji, M. (1989) A case of ocular toxocariasis with frosted vasculitis of the retina [in Japanese with English summary]. *Japanese Journal of Clinical Ophthalmology* **43,** 1369–1373.

Molk, R. (1982) Treatment of toxocaral optic neuritis. *Journal of Clinical Neuro-Ophthalmology* **2,** 109–112.

Molk, R. (1983) Ocular toxocariasis: a review of the literature. *Annals of Ophthalmology* **15,** 216–219.

Nagakura, K., Kanno, S., Tachibana, H., Kaneda, Y., Ohkido, M., Kondo, K. & Inoue, H. (1990) Serologic differentiation between *Toxocara canis* and *Toxocara cati* [letter]. *Journal of Infectious Diseases* **162,** 1418–1419.

Parker, II, D.W. & Shaver, R.P. (1996) Toxocariasis. pp. 1225–1235 *in* Pepose, J.S., Holland, G.N. & Wilhelmus, K.R. (*Eds*) *Ocular infection and immunity*. St Lois, Mosby.

Romeu, J., Roig, J., Bada, J.L., Riera, C. & Munoz, C. (1991) Adult human toxocariasis acquired by eating raw snails. *Journal of Infectious Diseases* **164,** 438.

Searl, S.S., Moazed, K., Albert, D.M. & Marcus, L.C. (1981) Ocular toxocariasis presenting as leukocoria in a patient with low ELISA titer to *Toxocara canis*. *Ophthalmology* **88,** 1302–1306.

Seki, F., Sakai, J., Rai, T., Nonaka, S., Usui, M., Tanabe, M. & Tsuji, M. (1990) Six cases of ocular toxocariasis [in Japanese]. *Atarashii Ganka [Journal of Eye]* **7,** 279–285.

Sharkey, J.A. & McKay, P.S. (1993) Ocular toxocariasis in a patient with repeatedly negative ELISA titre to *Toxocara canis*. *British Journal of Ophthalmology* **77,** 253–254.

Shields, J.A. (1984) Ocular toxocariasis. A review. *Survey of Ophthalmology* **28,** 361–381.

Tada, R. (1991) Antitoxocara antibodies in uveitis, and the diagnosis of ocular toxocariasis [in Japanese]. *Folia Ophthalmologica Japonica* **42,** 1035–1040.

Wilder, H.C. (1950) Nematode endophthalmitis. *Transactions of the American Academy of Ophthalmology and Otolaryngology* **55,** 99–109.

Wilkinson, C.P. & Welch, R.B. (1971) Intraocular toxocara. *American Journal of Ophthalmology* **71,** 921–930.

Yoshioka, H. (1966) Nematode endophthalmitis, possibly due to *Toxocara canis*. Report of a case. *Japanese Journal of Clinical Ophthalmology* **20,** 149–154.

Yuasa, T. (1994) Ocular *Toxocara canis* [in Japanese]. pp. 97–99 *in* Usui, M. & Ohno, S. (*Eds*) *Current encyclopedia of ophthalmology*. Tokyo, Nakayama-Shoten.

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