The comparision of IgG antibodies specific to *Toxocara spp*. among eosinophilic and non-eosinophilic groups

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SUMMARY

Toxocariasis is one of the most frequently reported helminth infections worldwide. Eosinophilia is a common finding of parasitic infections. This study assessed the levels of IgG antibodies specific to *Toxocara spp*. by ES-ELISA method in an eosinophilic (n=350) and non eosinophilic group (n=350). There were IgG antibodies specific to *Toxocara spp.* in 114 (32.6%) of the eosinophilic group and in 71 (20.3%) of the non-eosinophilic group (p<0.001). Toxocariasis may be an important problem in the region and should be kept in mind for patients with eosinophilia.

KEY WORDS: Toxocariasis, Eosinophilia, Aydın, Turkey

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INTRODUCTION

Toxocariasis is one of the most frequently reported helminth infections worldwide (Magnaval *et al.,* 2001). The most frequently seen helminth infection agents are *Toxocara cati (T.cati)* in cats and *Toxocara canis (T.canis)* in dogs, both are said to cause toxocariasis in human beings (Magnaval *et al.,* 2001, Parson *et al.,* 1987). The larvas in infective *Toxocara spp*. eggs, that infect human beings by oral route, pass the wall of the small intestine and enter the systemic circulation. Later they form granulomas first in the liver, then in lungs, brain and other organs (Hill *et al*., 1985).
Diagnosis of most parasitic infections can be made by detection of the agent itself or its eggs, but this is not the case for toxocariasis. Thus, serological methods gain importance for toxocariasis: ES-ELISA (Excretory secretory-enzyme linked immunosorbant assay) method with high sensitivity and specifity is especially one of the best alternatives (Magnaval *et al.,* 2001).
Eosinophils play a role in humoral immunity and also in the secretory immune system. One to three per cent of peripheral blood leucocytes consists of eosinophils. It is reported that the eosinophil count increases in allergic diseases, parasitic infections and oncologic diseases (Takamoto *et al.,* 1998; Rothenberg and Epstein, 1998; Behm and Ovington, 2000; Guy and Athens, 1998).
The aim of this study was to assess IgG type antibodies specific to *Toxocara spp*. in groups of eosinophilic and non-eosinophilic patients in Aydin, a city in western Turkey.

MATERIALS AND METHODS

The study was performed in Adnan Menderes University Practice and Research Hospital. Samples from patients attending the hospital for any medical problem during the period May-July 2004 were included in the study. The samples

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were supplied daily by the Biochemistry Department of Adnan Menderes University Practice and Research Hospital and were preserved at -20°C. The samples of patients were divided into two main groups, then three subgroups for the second group, according to eosinophil counts as below (Parson *et al.,* 1987).

1. Non-eosinophilic group (n=350): patients having ≤350/ml eosinophils.
2. Eosinophilic group (n=350): patients having ≥351/ml eosinophils.
   2.1. Mild eosinophilic group (n=296): 351-1500/ml eosinophils.
   2.2. Moderate eosinophilic group (n=44): 1501-5000/ml eosinophils.
   2.3. High eosinophilic group (n=10): more than 5000/ml eosinophils.

Antibodies to *Toxocara spp*. were assessed with in house ELISA using excretory/secretory (E/S) antigens (Ajayi *et al.,* 2000; Demirci *et al.,* 2002). Positive and negative control sera and Toxocara E/S antigens were kindly provided by Dr. H. Auer (Institute of Hygiene, University of Vienna).

For these tests previously worked-out chess-board titrations with different antigen and conjugate concentrations were performed to establish optimal test conditions. Tests were performed in duplicate. Briefly, the wells in microtiter plates were sensitized with the antigen overnight at 4°C. The plates were then given three washes each of 3 min in phosphate buffered saline (PBS pH 7.4) containing 0.05% Tween 20 (PBS Tween). All sera were tested in a 1:100 dilutions. Sera to be assayed were diluted in PBS Tween and 100 µl volumes were added to each well. After one hour incubation at 37°C the plates were washed five times with PBS Tween. The wells were then filled with 100 µl of anti-human IgG labeled with alkaline phosphatase conjugate (Sigma, A-3187) diluted 1:10.000 in PBS Tween, and incubated for one hour at 37°C. After five washings, 100 µl substrate (Sigma N-2765) was added to each well. The enzymic hydrolysis of substrate was stopped after 30 min by the addition of 100 µl NaOH. After 30 min the Optical density was measured with a spectrophotometer at 405 nm by Microplate Reader. Results were considered positive when the extinction value 6-8 negative control sera were raised with three times the standard deviation.

A statistical program was used to assess collected data. The descriptive statistics are shown by percentages and arithmetical means±standard deviation (minimum-maximum values). Chi-squared and Student t tests have been used in analytical assessment. The differences were considered to be statistically significant when the p value obtained was less than 0.05.

RESULTS

The mean age of the non-eosinophilic group was 41.96±17.12 (3-81) years; 242 (69.1%) of them were female and 108 (30.9%) were male. The mean age of the eosinophilic group was 40.87±20.62 (1-90) years; 203 (58.0%) of them were female and 147 (42.0%) were male.

The overall prevalence of IgG antibodies specific for *Toxocara spp.* was 26.4% (n=185). There were IgG antibodies specific for *Toxocara spp.* in 29.0% (n=74) of men and 24.9% (n=111) of women (p=0.239). There were IgG antibodies specific to *Toxocara spp.* in 114 (32.6%) of the eosinophilic group and 71 (20.3%) in the non-eosinophilic group (p<0.001). All of the detected antibodies in the eosinophilic group were belonged to mild eosinophilic group (38.5%; 114 of 296). No IgG antibodies specific to *Toxocara spp.* were detected in moderate or in high eosinophilic groups.

The IgG presence according to age groups for the mild eosinophilic group and non-eosinophilic count group is given in the Table 1. In our study, 15 (57.7%) out of 26 cases in 0-10 years of age and 31 (55.4%) out of 56 older than 61 years of age in the mild eosinophilic group had significantly higher specific antibodies for *Toxocara spp.* than other age groups (p=0.013) The highest prevalence was also observed in the 0-10 years age group in the non-eosinophilic group with 6 out of 16 cases (37.5%), but the difference was not statistically significant.

DISCUSSION

Although it is known that infectious, malignant and allergic diseases can cause an increase in eosinophil counts in peripheral blood, it is accepted that the most common eosinophilia cause worldwide is parasitic infection. The common parasitic diseases causing strong eosinophilia are schistosomiasis, filariasis, trichinosis, toxocaria-

TABLE 1 - *IgG antibodies specific to Toxocara spp. presence according to age groups in non-eosinophilic and mild eosinophilic groups.*

Group (Eosinophil count/ml)	*Age groups*	*IgG antibody specific to Toxocara spp.*		*Total count n (%)**
		*Present n. (%**)*	*Absent n. (%**)*	
Mild eosinophilic group (351-1500)	0-10	15 (57.7)	11 (42.3)	26 (8.8)
	11-20	12 (33.3)	24 (66.7)	36 (12.2)
	21-30	11 (25.6)	32 (74.4)	43 (14.5)
	31-40	12 (36.4)	21 (63.6)	33 (11.1)
	41-50	18 (34.6)	34 (65.4)	52 (17.6)
	51-60	15 (30.0)	35 (70.0)	50 (16.9)
	≥61	31 (55.4)	25 (44.6)	56 (18.9)
	Total	114 (38.5)	182 (61.5)	296 (100.0)
Non-eosinophilic group (<350)	0-10	6 (37.5)	10 (62.5)	16 (4.6)
	11-20	5 (17.2)	24 (82.8)	29 (8.3)
	21-30	9 (22.0)	32 (78.0)	41 (11.7)
	31-40	7 (10.1)	62 (89.9)	69 (19.7)
	41-50	16 (18.6)	70 (81.4)	86 (24.6)
	51-60	13 (22.8)	44 (77.2)	57 (16.3)
	≥61	15 (28.8)	37 (71.2)	52 (14.9)
	Total	71 (20.3)	279 (79.7)	350 (100.0)

*Column percentage; **Row percentage.

sis, and fasciolosis (Takamoto *et al.,* 1998; Chusid, 1999; Parson *et al.,* 1987). In the current study, the overall prevalence of IgG antibodies specific for *Toxocara spp*. was 26.4%. In different territories worldwide, detection rates of antibodies specific to *Toxocara spp.* have a wide range between 5.1% and 76.6% (Magnaval *et al.,* 2001; Ljungström and Van Knapen, 1989; Park *et al*., 2002; Sadjjadi *et al.,* 2000; Fan *et al.,* 2004). In Turkey, the seroprevalence of *Toxocara* is reported to be between 28.57% and 51.35% in various studies (Gungor *et al.*, 1999; Oguzturk and Saygı, 2002). There is only one study on *Toxocara* epidemiology in our region. In this study specific IgG antibody against *T.canis* were assessed with E/S ELISA in 100 epileptic patients and 50 healthy volunteers. No difference was found between seropositivity rates of patients (12%) and control groups (5.9%). They found no significant relationship between the occurrence of pica and *Toxocara* seropositivity rate. They also found no significant relationship in *Toxocara* seropositivity between those living in rural and urban areas. This might be linked to the fact that most people work in agriculture in both urban and rural areas in Aydın province (Akyol *et al.*, 2007).
In cases with high total serum IgE and/or eosinophil levels, toxocariasis should be entertained in the differential diagnosis (Magnaval *et al.,* 2001). In a Turkish study, antibodies specific to *Toxocara* were detected in 29.1% patients with eosinophilia (Demirci *et al.,* 2002). In the current study, 32.6% of the eosinophilic group and 20,3% of the non-eosinophilic group had specific antibodies to *Toxocara*. As all of the detected antibodies in the eosinophilic group belonged to mild the eosinophilic group, it was thought that toxocariasis should be especially taken into account in patients with mild eosinophilia. There are different results declared by researchers on the relation of the frequency of *Toxocara* and age. Some of them found no significant change with age, whereas others claimed that *Toxocara* is more frequent in childhood (Takamoto *et al.,* 1998; Ajayi *et al.,* 2000; Anaruma Filho *et al.,* 2002; Aguiar-Santos *et al.,* 2004). It is known that toxocariasis is transmitted orally by taking the infective eggs which are found in soil contaminated with feces of infected cats and dogs. Thus, individuals having high risk of a contact with soil contaminated with cat and dog feces have higher antibody detection rates (Magnaval *et al.,* 2001). In the current study, the high antibody rates detected in the 0-10 age group might be due to contamination of playfields with infected cat and dog feces. Gurel S. *et al.* detected *Toxocara spp.* egg contamination in 18.9% of playfields in Aydin which supports this

hypothesis (Gürel *et al.,* 2005). Additionally, the *Toxocara* seropositivity detected in over 61 years mild eosinophilic group was higher than the other groups. No data could be found related to this subject in literature searches. But when the social situation of the study region is taken into account, most individuals in this age group could probably have a past in a rural region, so they could probably have had a contact with contaminated soil which is a risk factor for toxocariasis. So, it is thought that the results in the elderly reflect past infections. The current study has some limitations to obtain the real prevalence rate of toxocarisasis in the community because the study was performed among patients and the method is not a reference one. However, this study may give an idea of the presence of toxocariasis in our region for which there are only a few similar studies. This study may also help in carrying out a larger scale investigation for detecting real prevalence rates. In conclusion, toxocariasis may be an important problem and should be kept in mind for patients with eosinophilia.

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