Diagnostic Importance of Serological Methods and Eosinophilia in Tissue Parasites

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ABSTRACT

This case-control study was carried out to investigate whether eosinophilia is a common finding in parasitic diseases and to determine the prevalence of the parasitic diseases in definitive groups. Toxocariasis and fasciolosis were investigated in patients with eosinophilia, patients with non-eosinophilia, and healthy controls. All cases were analyzed for antibodies against Toxocara canis and Fasciola hepatica by modified enzyme-linked immunosorbent assay (ELISA) employing, as antigen, excretory/secretory products of Toxocara and Fasciola (ES-ELISA). Seropositivity was determined in 39 (29.1%) and 12 (8.9%) toxocariasis and fasciolosis patients with eosinophilia respectively. The seropositivity was found in 26 (19.4%) and 5 (3.7%) toxocariasis and fasciolosis patients without eosinophilia respectively. The seropositivity was found in 13 (15.5%) and 1 (1.2%) toxocariasis and fasciolosis patients in the control group. The total seropositivity in both toxocariasis and fasciolosis in the eosinophilic group (8.5%) increased significantly compared to patients without eosinophilia (23.1%) and healthy controls (6.6%) ($\chi^2$:6.343, p<0.05 and $\chi^2$:10.293, p<0.01). Eosinophilia correlated with antibodies against Toxocara and F. hepatica. The results suggest that serological methods can be used for diagnosis of parasitic infections in tissue. Thus, the actual prevalence of tissue parasites can be revealed particularly in developing and emerging countries.

Key words: Enzyme-linked immunosorbent assay; Serodiagnosis; Eosinophilia; Fasciolosis; Toxocariasis; Case-control studies; Turkey

INTRODUCTION

Eosinophilia is a well-known response of the host to invasion by parasites. The most common cause of eosinophilia is helminthic infections (1). High levels of eosinophils may be found in patients with trichinosis, ascariasis, filariasis, schistosomiasis, toxocariasis, and fasciolosis (1-3).

Although routine examination of parasite in stools has been widely carried out in patients with eosinophilia, tissue parasites received insufficient attention in diagnosis (4). Although serologic tests for the parasite are not routinely used in the diagnosis of parasitic diseases, such as toxocariasis (5) and fasciolosis (6), such tests are most useful in parasitic diseases with particular asymptomatic or indistinguishable clinical manifestations (2).

We investigated whether eosinophilia is a common finding of parasitic diseases in our region. In addition, we determined the seroprevalence of these parasitic diseases in definitive groups.

MATERIALS AND METHODS

In this case-control study, 134 eosinophilic patients, 134 non-eosinophilic patients, and 84 healthy persons were enrolled. The eosinophilic patients included cases who were referred to the Süleyman Demirel University.
Hospital for skin lesions (47), abdominal pain (39), respiratory symptoms (17), rheumatisms, and hypertension (31). The non-eosinophilic patients included cases of skin lesions (42), abdominal pain (43), respiratory symptoms (21), rheumatisms, and hypertension (28). These cases and the healthy controls were randomly selected, but age and sex were controlled. Cases with malignancy and/or parasite egg or cyst in stool were excluded from the study.

A level of \( \leq 350 \) eosinophils/µL was considered normal, 350-1,500 eosinophils/µL mild, 1,500-5,000 eosinophils/µL moderate, and >5,000 eosinophils/µL significant (1).

The mean age of eosinophilic patients, non-eosinophilic patients, and healthy control group was 39.4±16.44 years (female/male 76/58), 40.1±16.21 years (female/male 73/61), and 43.1±15.84 years (female/male 50/34) respectively.

All cases were examined for antibodies against *Toxocara* and *Fasciola* by modifying the ELISA method according to Glickman et al. (5) and Espino et al. (6). Briefly, adults of *F. hepatica* were incubated at 37 °C for three hours in phosphate-buffered saline containing 0.8 mol/L phenylmethylsulfonyl fluoride, 400 U of aprotinin per mL, and 0.1 mM dithiothreitol (one worm/5 mL) (Sigma Chemical, St. Louis, USA). *Toxocara* human IgG (Sigma) was used at 1:10,000 dilution. The substrate was 4-nitrophenyl phosphate disodium salt (Merck, Darmstadt, Germany). Plates were read on a microplate reader (Bio-Tek, ultramicroplate reader ELX 808, Winooski, USA) at an absorbence of 405 nm. The cut-off point was calculated as the average of absorbence values of negative sera plus three standard deviations (5,6).

Chi-square test was performed to confirm the difference between groups. The level of significance selected was \( p<0.05 \).

### RESULTS

In this study, the highest seropositivity rates of toxocariasis and fasciolosis were determined in the eosinophilic group compared to those of non-eosinophilic patients and healthy controls (Table).

The seropositivity of both toxocariasis and fasciolosis in the eosinophilic group was significantly higher compared to that of the healthy controls (\( \chi^2:4.556 \) and 4.253 respectively, \( p<0.05 \) for all). No significant statistical differences in the seropositivity of toxocariasis and fasciolosis were found between both patients with eosinophilia and non-eosinophilia, and between non-eosinophilic group and healthy control group. The total seropositivity of both toxocariasis and fasciolosis in the eosinophilic group was significantly higher compared to that of the non-eosinophilic and healthy control groups (\( \chi^2:6.343, p<0.05 \) and \( \chi^2:10.293, p<0.01 \) respectively).

Cross-reactivity was found in five sera of eosinophilic patients and in two sera of non-eosinophilic patients. We evaluated 4 of the 7 cross-reaction-positive patients. The other three cross-reaction-positive cases could not be investigated further due to various reasons. We observed that two cases were concordant with fasciolosis. In one case, we determined *Taenia saginata* proglottids and eggs by examining stool, and in the other case, we did not determine any signs and symptoms of

### Table. Eosinophilia and seropositivity in study groups

<table>
<thead>
<tr>
<th>Eosinophil level/ study group</th>
<th>Eosinophilia</th>
<th>Toxocariasis</th>
<th>Fasciolosis</th>
<th>Total positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>350-1,500</td>
<td>124</td>
<td>92.5</td>
<td>35</td>
<td>28.2</td>
</tr>
<tr>
<td>1,500-5,000</td>
<td>8</td>
<td>6.0</td>
<td>3</td>
<td>37.5</td>
</tr>
<tr>
<td>&gt;5,000</td>
<td>2</td>
<td>1.5</td>
<td>1</td>
<td>50.0</td>
</tr>
<tr>
<td>Eosinophilic patient</td>
<td>134</td>
<td>100</td>
<td>39</td>
<td>29.1b</td>
</tr>
<tr>
<td>Non-eosinophilic patient</td>
<td>134</td>
<td>-</td>
<td>26</td>
<td>19.4</td>
</tr>
<tr>
<td>Healthy control</td>
<td>84</td>
<td>-</td>
<td>13</td>
<td>15.5b</td>
</tr>
</tbody>
</table>

a,b Significant statistical difference between groups

larvae were incubated at 37 °C for one week in RPMI 1640 medium (Sigma) containing 100 U/mL penicillin and 250 µg/mL streptomycin (Pfizer, Istanbul, Turkey). The suspension containing excretory-secretory antigen of *F. hepatica* (ESAFh) and *Toxocara* (ESA(Tc) was centrifuged at 4 °C (13,000xG) for two hours and was filtered by a 0.2-µm pore-size filter.

ESAFh and ESA(Tc) were coated to immunoplate (Nunc, Roskilde, Denmark and Costar, Schiphol Rijk, The Netherlands) at concentrations of 12.8 µg/mL and 95 µg/mL respectively. Human sera were used at 1:100 dilution, and alkaline phosphatase-conjugated anti-
these parasitic diseases so that this case may be infected beforehand with any parasitic diseases.

**DISCUSSION**

Our data show that eosinophilia correlated with antibodies against *T. canis* and *F. hepatica*. The findings suggest that these parasitic diseases may be endemic in our region, and it is expected that these parasitic diseases were not considered in eosinophilic or non-eosinophilic patients in our region up to the present time. Although eosinophilia particularly suggests the presence of a helminth, the absence of eosinophilia cannot exclude these parasites (2).

Toxocariasis, an infection caused by the larvae of *Toxocara* sp., may involve many organs, but pulmonary symptoms, such as cough and wheezing and allergic symptoms, are seen in more than 80% of patients (7). Diagnosis of toxocariasis is difficult since clinical and laboratory data provide insufficient evidence. Diagnosis of toxocariasis depends on serologic methods (5). ELISA is reported to have 78.3% specificity and 92.0% sensitivity in diagnosis of toxocariasis (5). In France, seropositivity of toxocariasis was 49% in eosinophilic and asymptomatic patients after visiting tropical countries (2). In Egypt, seropositivity in eosinophilic patients was determined to be 20% (8). Although eosinophilia particularly suggests the presence of a helminth, the absence of eosinophilia cannot exclude these parasites (2).

**DEFINITIVE DIAGNOSIS OF FASCIOLIOSIS**

Definitive diagnosis of fasciolosis depends on serologic tests and/or demonstration of *F. hepatica* eggs in stool and/or bile samples (10). Serologic studies are now the main diagnostic methods and allow diagnosis of the disease even in the acute stage, even before the parasite eggs can be identified in faeces. ELISA is a rapid and sensitive test and has 95-100% specificity and 93-97% sensitivity (6,11,12). Fasciolosis was reported in many Mediterranean countries (10,11). The seroprevalence of fasciolosis was detected in 12-56% of eosinophilic patients in Egypt, and the importance of eosinophilia was emphasized in diagnosis of fasciolosis (8). In our country, no sero-epidemiological study has been carried out.

In addition, the unsatisfactory sensitivity of immunodiagnostic tests for parasitic diseases still poses problems, and a certain number of false-positive results or cross-reactions are being expected. Cross-reactivity may be found among helminthic infections with a low seropositivity value (3,5-7). In this study, cross-reactivity was detected in 7 cases of toxocariasis and fasciolosis. These cross-reactivities may reduce the development of specific antigens of species.

In this study, seropositivities of fasciolosis and toxocariasis were determined in Isparta region (southwestern part of Turkey known as a region of lakes) for the first time. We also considered that this region may be a focus for fasciolosis because its climate is appropriate for *Lymnaea truncatula*, the intermediate host of *F. hepatica*.

We conclude that parasitic infections should be considered an important cause of eosinophilia. Serological methods should be used for exposing diagnosis, survival, and prevalence of tissue parasitic infections. Thus, the actual prevalence of tissue parasites can be revealed particularly in developing countries.

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**REFERENCES**


