

Seroprevalence of Toxocariasis among Healthy People with Eosinophilia

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Abstract : The aim of this study is to determine the *Toxocara* seropositive rate among healthy people with eosinophilia. A total of 97 people residing in Seoul who were healthy and whose blood eosinophilia was over 10%, as shown by regular health check-ups in 2004, were subjected to this study. Their sera were tested by immunoblotting and ELISA with the antigen of larval *Toxocara canis* excretory-secretory (ES) protein. Sixty-five sera were band-positive (67.0%). The seropositive control sera were positive to band sizes of 66 kDa, 56 kDa, 32 kDa, and 13 kDa. In ELISA, 63 sera (65.0%) were positive to *T. canis* ES protein. There was no significant correlation between the IgG ELISA titer and the level of eosinophilia ($r = 0.156$, $P = 0.156$). As there were insufficient data to determine whether there were cross-reactions with other helminthic infections, or whether atopy occurred, further studies are required to verify the cause of the seropositive reactions against *T. canis* ES antigen. Toxocariasis seropositivity is suggested to be the major cause of eosinophilia, since the *Toxocara* seroprevalence among Korean rural adults was shown to be approximately 5%.

Key words: *Toxocara canis*, Toxocariasis, Eosinophilia, Immunoblotting

INTRODUCTION

It is known that eosinophilia occurs when humans are infected with *Toxocara canis* or *Toxocara cati*. The *Toxocara* seropositive rate varies in different countries and environments. In Europe, the seropositive rate is 2 to 5 percent in apparently healthy urban adults, while the rate is 14.2% to 37% in rural areas [1]. In the Republic of Korea, where the prevalence of toxocariasis in dogs is 0.9% ($n = 662$), the toxocariasis seroprevalence among rural adults is approximately 5% [2,3]. In India, of 94 people from the general population and 30 patients with clinically suspected toxocariasis, 6 (6.4%) and 7 (23.3%), respectively, were seropositive to *Toxocara* excretory-secretory (ES) antigen [4]. In Slovenia, of 239 patients with ocular symptoms, 67 (28%) were *Toxocara* seropositive [5]. In Argentina, of 114 rural residents in estancias (cattle- or sheep-breeding ranches), 36 (31.6%) were seropositive [6]. It has also been reported that asthma patients showed a seropositive rate of 99.3% to *Toxocara* ES antigen [7]. In Korea, out of 127 sera from patients with eosinophilia (more than 500 / μ l or more than or equal to 10% WBC) who visited the hospital, 70 sera (68%) were positive to ES antigen and patients with lesions in the liver showed higher serum eosinophil cationic pro-

tein (ECP) values [8]. However, there is no data on the seropositive rate of toxocariasis in the general population with eosinophilia (more than or equal to 10% WBC). The present study was performed to determine what proportion of the healthy general population with eosinophilia shows seropositive against *Toxocara* ES antigen by ELISA and immunoblotting.

MATERIALS AND METHODS

For the preparation of the ES antigen, eggs were recovered from the uterus of a *T. canis* adult female. They were incubated in 0.5 % formalin for 1 month at room temperature. The eggshells were broken by a homogenizer. Live larvae were collected using a Baermann's apparatus. The larvae were kept and stored in a culture medium (RPMI 1640 buffered to pH 7.2 with 23 mM L-glutamine and 100 μ g / ml gentamicin) at 37°C in a CO₂ incubator. Amphotericin B 1 : 1,000 (v / v) was added to the culture media to prevent fungal contamination. The supernatant was collected weekly, concentrated by ultrafiltration (10,000 KdMr exclusion limit, Amicon, Millipore, San Francisco California, USA), and used as the ES antigen. The protein concentration of the ES antigen was measured using the Lowry method. A total of 97 sera showing higher than 10% eosinophilia were collected from apparently healthy adults in Seoul with no reports of any symptoms. These sera were provided by the Korea Association of Health

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Promotion during the regular screening in 2004. They were stored at -70°C until use. The providers of the sera gave no additional information on any parasitic, allergic, rheumatoid diseases, or cancer.

For the immunoblot analysis, the ES-Ag preparations were separated by 7.5-15% SDS-PAGE and transferred onto a PVDF membrane (Amersham Pharmacia Biotech Inc., Piscataway, New Jersey, USA) in a semi-blotter (Hoffer, San Francisco, California, USA). Transferred membranes were treated with casein buffer for 1 hr to remove non-specific reactions. The membranes were cut into 2 mm-wide strips. The strips were then incubated overnight with sera diluted at 1 : 100 and then washed 3 times with phosphate-buffered saline (PBS / T, pH 7.4, 0.15 M NaCl, 0.05% Tween 20). Goat peroxidase-conjugated anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania, USA) diluted at 1 : 1,000 PBS / T was used to detect the immunoreaction. Strips were washed 3 times with PBS / T. As the enzyme substrate, 0.005% 4-chloro-1-naphthol (Sigma Co., St. Louis, Missouri, USA), was used.

For the ES IgG ELISA, microtiter plates (Costar-Corning, Cambridge, California, USA) were coated overnight at 4°C with 200 μl of ES antigen solution in carbonate-bicarbonate buffer (pH 9.6) containing 10 $\mu\text{g}/\text{ml}$ proteins. Each serum was diluted at 1 : 100 in casein buffer. Two hundred μl of diluted sera were added to the wells and incubated for 2 hr at 37°C . Peroxidase-conjugated anti-human IgG (Cappel, West Chester, Pennsylvania, USA) was diluted at 1 : 5,000. Each 200 μl was added to the well and incubated for 2 hr at 37°C . The substrate for color reaction was 0.05% (w.v) *o*-phenylenediamine with 3% (v / v) H_2O_2 . The reaction was stopped with 25 μl of 8 M H_2SO_4 . The absorbance at 490 nm was recorded with a microtiter plate reader (Bio-Rad M3550, Richmond, California, USA). The positive criterion was set at 0.8 in average or to 0.9 in each trial.

Positive and negative reference sera for ELISA and immunoblotting were from previous reports [3]. The positive and negative criteria were calibrated with those reference sera. Pearson product-moment correlation was performed using the statistical package, dBSTAT 4.0 available from <http://dbstat.com>.

RESULTS

In the immunoblotting, 4 major bands were observed; 13 kDa, 32 kDa, 56 kDa, 66 kDa from 65 sera. The occurrence of at least 1 major band was denoted as being immunoblot positive (Fig. 1). Combinations of the positive band patterns and

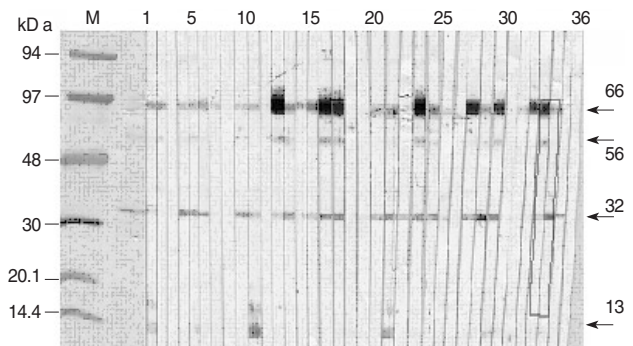


Fig. 1. Band patterns after immunoblotting of human sera with eosinophilia against *Toxocara* excretory-secretory antigen. M: protein maker, 1-32: Eosinophilia sera, 33-34: Positive controls, 35: Other sample serum, 36: Negative control.

the number of corresponding sera (in parentheses) were as follows: 13 kDa (2 sera), 32 kDa (8), 56 kDa (0), 66 kDa (10), 66 + 13 kDa (1), 66 + 32 kDa (19), 66 + 56 kDa (1), 66 + 56 + 32 kDa (10), 66 + 32 + 13 kDa (4), 66 + 56 + 32 + 13 kDa (1), 32 + 13 kDa (3), and 32 + 56 kDa (7). Of these combinations, 66 kDa and 32 kDa were most frequent bands. The number of sera positive to 13 kDa was 10 (15.4%), to 32 kDa was 52 (80%), to 56 kDa was 19 (29.2%), and to 66 kDa was 46 (70.8%). The number of sera positive to both 66 kDa and 32 kDa was 34 (35.1%). With ELISA, 63 sera (65.0%) were positive to *Toxocara* ES. There was no significant correlation between the IgG ELISA titer and the degree of eosinophilia ($r = 0.156$, $P = 0.156$). The number of sera positive to both immunoblot and ELISA was 58 (60.0%), the number negative to both was 27 (27.8%), the number of immunoblot positive and ELISA negative was 7 (7.2%), and the number of immunoblot negative and ELISA positive was 5 (5.2%).

DISCUSSION

Although it is known that eosinophilia is associated with toxocarasis, there have been few serological studies on the relationship between toxocarasis and eosinophilia. In a previous study, 14 (93.3%) of 15 patients with eosinophilia showed positive reactions in a *Toxocara* serologic test [7]. In the present study, the immunoblot positive rate was 67%, and that by ELISA 65%. These are comparable with the study of Kwon et al. [8]. The difference may have been due to a difference in the subjects assessed. The present study targeted apparently healthy adults, whereas Kwon et al. [8] studied patients who visited a University Hospital.

The causes of eosinophilia are known to be mainly allergic

diseases, parasitic diseases, cancer, and rheumatic diseases. Among the parasitic diseases, toxocariasis may be the most common cause of eosinophilia. The sera used in the present study were from people without any clinical symptoms whose health status was regularly checked with a comprehensive medical test. The cross reaction to other helminthic infections is negligible [3]. In the above results, the seropositive rate for both the immunoblot and ELISA at 60.0% was very high in comparison with that of the previous data from apparently healthy rural adults (5%). There were 5 sera (5.2%) that were immunoblot negative and ELISA positive. These may be false positives in ELISA results. There were also 7 (7.2%) sera that were immunoblot positive but ELISA negative. These may be false negatives in ELISA or they may have originated from a low titer, although the antibody reaction is specific to certain bands. To get more accurate information on the active toxocariasis among people with eosinophilia, further comparative studies with enough numbers of the reference group are needed. Also, the possibility of cross reactions with other helminthic diseases should be eliminated.

In the present study, the immunoblotting band pattern was different from that of the previous studies. Maizel et al. [9] reported 32, 55, 70, 120 kDa and 400 kDa bands. Magnaval et al. [10] mentioned 2 groups of bands: a high molecular weight group 132, 147, and 200 kDa, and a low molecular weight group 24, 28, 30, and 35 kDa. The low molecular weight bands readily cross-react with other parasitic nematode infections [10]. Nunes et al. [11] reported 5 groups: bands larger than 205 kDa, bands near 205 kDa, 116-97 kDa, 55-50 kDa, and 35-29 kDa. In the present study, high molecular bands above 66 kDa could not be detected. The different immunoblotting band patterns may have originated from differences in the ways the antigen was acquired, different laboratory techniques, variability of the epitopes of antigens or cross reaction with other helminthic infections. To overcome this variability, selection of a specific antigen and production of recombinant antigens is necessary. Double-sandwich ELISA with *Toxocara* specific antigen could also be applied for better screening of toxocariasis [12].

No clinical information on the symptoms, including atopy or allergies of the serum donors, was collected, since they were apparently healthy people. To detect a current active infection of toxocariasis, IgE needs to be tested by ELISA and an assessment of eosinophil cationic protein (ECP) is required [7,13]. People that test positive may have covert toxocariasis, so that a primary health care may need to include screening for toxocariasis among those

with eosinophilia [14]. This would prevent misdiagnosis of toxocariasis as idiopathic hypereosinophilic syndrome or cancer, which may result in inappropriate clinical interventions [15].

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