

Relationship between *Toxocara* seropositivity and allergic rhinitis

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ABSTRACT

Background: Allergic rhinitis is the most common chronic disease in the upper respiratory tract, requiring better diagnostic methodology to counteract its onslaught. The purpose of this study was to investigate the association between *Toxocara* seropositivity and allergic rhinitis.

Methods: Sixty-four consecutive patients with allergic rhinitis and 61 healthy volunteers in the control group were included in this study. The diagnosis of allergic rhinitis was defined by positive epicutaneous prick test. The diagnosis of toxocariasis was established by IgG and specific IgE antibodies against *Toxocara* by ELISA method. All cases were examined for intestinal parasites also. The seropositive cases with *Toxocara* were reevaluated for symptoms and signs of toxocariasis.

Results: Higher toxocariasis seropositivity (ELISA IgG; 28.1%) rate was determined in the patients with allergic rhinitis and compared with the control groups (11.5%; $p < 0.05$). Specific IgE seropositivity (17.2%) was high for the patients with allergic rhinitis compared with the control group (3.3%; $p < 0.05$).

Conclusion: Our results show that there was a possible causal relationship between *Toxocara* infection and allergic rhinitis. Patients with allergic rhinitis from a region with high prevalence for this parasitic infection should be evaluated for *Toxocara* infection.

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Key words: Allergic rhinitis, allergic sensitization, atopy, TH2, risk factors, tissue parasites, toxocariasis

Allergic rhinitis is the most common chronic disease in the upper respiratory tract, requiring better diagnostic methodology to counteract its onslaught.¹ The essential components of allergic reactions include allergens—IgE antibodies directed at antigenic determinants on the allergens. Inhalants are the most common allergens. Pollens are the most apparent of inhalant allergens, followed by dust mites, mold spores, and animal dander. A wide variety of food allergens are increasingly involved in the etiology of allergic disease of the upper respiratory tract. Biological agents, drugs, insect stings, and animal antiserum are some of the many allergens identified in the pathogenesis of the allergic reactions in humans.²

Parasitic infections may lead to allergic reactions in humans.^{3–5} Although the stool examination for parasites has been widely performed with allergic diseases, insufficient attention has been given to tissue parasites in diagnosis.⁶ Human toxocariasis is a worldwide helminthozoonosis that may elicit syndromes including urticaria, asthma, and other allergic disorders.^{7,8} Although, toxocariasis is known to cause many allergic reactions, according to our literature knowledge, there is no clear investigation concerning toxocariasis and allergic rhinitis in the literature. The purpose of this study was to investigate the association between *Toxocara* seropositivity and allergic rhinitis.

MATERIALS AND METHODS

Sixty-four consecutive patients with allergic rhinitis were included in this study. The main symptoms of the patients

were sneezing; watery rhinorrhea; itching in the nose, eyes, and palate; nasal blockage; and rhinorrhea. The diagnosis of allergic rhinitis was defined by history, endoscopic examination, and positive epicutaneous prick test (Multitest; Center Laboratories, Port Washington, NY). The diagnosis of toxocariasis was established by IgG and specific IgE antibodies against *Toxocara* by modified ELISA method according to Glickman *et al.*⁹ In addition, all cases were examined for intestinal parasites. The seropositive cases with *Toxocara* were reevaluated for symptoms and signs of toxocariasis.

Sixty-one healthy volunteers were included in the control group. This group consisted of patients with only nasal obstruction due to septum deviation. They had no history and examination findings for allergy. Also, epicutaneous prick test was negative in all patients.

Twenty-five (39%) of patients in the study group and 23 (38%) healthy volunteers were from the countryside. In addition, approximately one-half of urban persons from both groups were closely related to rural area. There was no outdoor park worker or veterinarian in either group. Only nine patients in the study group and eight persons in the control group graduated from college.

Epicutaneous Prick Test

The test allergens (Multitest) were supplied in 1:10 w/v strength with 50% glycerine as solvent. The negative control was used in 50% glycerine. A special applicator soaked into the dipwell tray of allergens was administered on the flexor surface of the forearm. The test was assessed at 20 minutes. The exact size of the wheal was measured and recorded. A growth of 3 mm in diameter in excess of the negative control was considered a positive result, because this diameter provides acceptable sensitivity (75–95%) and specificity (30–60%).^{10–12}

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Serum Collection

Five milliliters of blood was taken from each patient; sera were separated, labeled, and stored at -20°C until used.

Excretory-Secretory Antigen (ESA) Preparation

Briefly, *Toxocara canis* larvae were incubated at 37°C for 1 week in RPMI-1640 medium (Sigma Chemical, St. Louis, MO) containing 100 U/mL of penicillin (Pfizer, Istanbul, Turkey) and 250 $\mu\text{g/mL}$ of streptomycin (Pfizer). The suspension containing the ESA of *Toxocara* (ESATc) was centrifuged at 4°C ($13,000 \times g$) for 2 hours and was filtered by a $0.2\text{-}\mu\text{m}$ pore size filter.

ELISA Procedure (for IgG and IgE)

ESATc was coated to immunoplate (Costar-High binding immunoplate, Schiphol-Rijk, The Netherlands) at a concentration of 12.8 and 95 $\mu\text{g/mL}$, respectively. Human sera were used at a 1:100 dilution, and alkaline phosphatase-conjugated anti-human IgG and anti-human IgE (Sigma Chemical) was used at 1:10,000 and 1:500 dilutions. The substrate was 4-nitrophenyl phosphate disodium salt (Merck, Darmstadt, Germany). Plates were read on a microplate reader (Ultramicroplate Reader EL_x 808; Bio-Tek Instruments, Winooski, VT) at an absorbance of 405 nm. The cutoff point was calculated as the average of the absorbance values of negative sera plus 3 SD.

Stool Examination

Stool samples were examined for eggs, cysts, and/or trophozoites of intestinal parasites by a wet mount of fresh specimen by direct saline and Lugol preparation and by a formalin-ethyl acetate concentration technique.⁶ Statistical analysis was done by Fisher's exact chi-square test and student *t*-test.

RESULTS

In the study group there were 22 male patients and 42 female patients. The ages ranged between 12 and 46 years (average, 23.6 ± 9.8 years). In the control group, there were 21 male subjects and 40 female subjects. The ages ranged between 11 and 46 years (average, 22.7 ± 8.9 years). There is no difference between the two groups regarding age and sex ($p = 0.5588$).

Higher toxocariasis seropositivity (ELISA IgG; 28.1%) rate was determined in the patients with allergic rhinitis and compared with the control groups (11.5%; $p = 0.0253$; Table 1). Specific IgE seropositivity (17.2%) was high in the patients with allergic rhinitis compared with the control group (3.3%; $p = 0.0166$; Table 2). In the stool examination, we determined

one cyst of *Giardia intestinalis* and two cysts of *Entamoeba coli* in three patients with allergic rhinitis, but, there was no toxocariasis seropositivity in these patients.

Epicutaneous prick test was positive in all patients in the study group. The healthy control subjects had no allergy in their medical history and no reactivity in the epicutaneous prick test. The results of epicutaneous prick test found multiple allergens effective in 51 (79.7%) of 64 patients in the study group, and there was a single allergen reactivity in 13 cases. The most common reactions were a mixture of grass pollens in 47 (73.4%) of 64 patients, followed by mites (38 patients, 59.4%), tree mix (22 patients, 34.4%), weed mix (18 patients, 28.1%), mold mix (16 patients, 25.0%), and mixed epidermals (8 patients, 12.5%).

DISCUSSION

Our data has shown that toxocariasis may be an etiologic factor in patients with allergic rhinitis. *Toxocara* is a round-worm and a common parasite of dogs (*T. canis*) and cats (*Toxocara cati*). Toxocariasis is an infection caused by the larvae of *Toxocara* sp., which may involve many organs. Pulmonary and allergic symptoms are seen in $>80\%$ of the patients with toxocariasis. In the course of toxocariasis, many ESAs that were not fully identified may cause antigenic stimulation.^{7,13} Diagnosis of toxocariasis is difficult because clinical and laboratory data provide insufficient evidence for the diagnosis. Currently, diagnosis of toxocariasis depends on serological methods. The diagnosis of toxocariasis by using ELISA test has a specificity of 78.3% and a sensitivity of 92.0%.⁹ In addition to the still unsatisfactory sensitivity of immunodiagnostic tests for parasitic diseases, specificity of the reactions poses problems, and a certain number of false positive results or cross-reactions have to be expected. It was reported that cross-reactivity might be found in low seropositivity values among ascariasis, taeniasis, schistosomiasis, toxoplasmosis, fasciolosis, and cystic echinococcosis.^{9,13,14} In this study group, we detected no parasites in the stool, and toxoplasmosis, fasciolosis, and cystic echinococcosis serology were negative by ELISA tests in these patients. Clinical and laboratory evaluation may be useful for diagnosis for confirming the cross-reactivities.

The original description by Coombs and Gell of a specific immune (IgE) mechanism underlying symptoms induced by allergens in the different target organs, for a long time, has guided our understanding of the pathophysiology of allergic diseases such as rhinitis, asthma, conjunctivitis, urticaria-angioedema, and atopic eczema.¹⁵ Although different allergens are involved in the upper airway allergic responses, parasitic diseases may be another cause of allergy.³⁻⁵

Table 1 IgG seropositivity in the study groups

Study Groups	<i>Toxocara</i> ELISA IgG ⁺	<i>Toxocara</i> ELISA IgG ⁻
Allergic rhinitis	18 (28.1%)	46 (71.9%)
Control	7 (11.5%)	54 (88.5%)
Fisher's exact test ($p = 0.0253$).		

Table 2 Specific IgE seropositivity in the study groups

Study Groups	<i>Toxocara</i> ELISA IgE ⁺	<i>Toxocara</i> ELISA IgE ⁻
Allergic rhinitis	11 (17.2%)	53 (82.8%)
Control	2 (3.3%)	59 (96.7%)
Fisher's Exact Test ($p = 0.0166$).		

The association between toxocariasis and allergic disorders was described in some studies. Buijs *et al.*¹⁶ studied children with toxocariasis and found a significant association with allergic disorders such as asthma and also with IgE specific to inhaled allergens. They reported that parasitic infection, including *Toxocara* infection, causes a nonspecific stimulation of quiescent allergic disorders in atopic children. Larval antigens encourage Th0 cells to develop Th2 cells, IL-4 (which stimulates IgE production by B lymphocytes), and IL-5 (which stimulates the production and maturation of eosinophils). The ability of *Toxocara* larvae to survive in their hosts for months stimulates Th2 cells and the consequent production of IgE for a long time. Oteifa *et al.*⁵ reported that children with allergic bronchial asthma or chronic urticaria showed a significant increase in specific IgE for *Toxocara* infection when compared with controls. They concluded that a contribution of toxocariasis to the allergic sensitization was suggested. One other mechanism may be possible in which this immunopathologic reaction serum IgG antibodies interact with an antigen (ESATc has been suggested as the antigen), forming an antigen-antibody complex that aggregates in and near blood vessels, inducing inflammation and leading to vascular damage and thrombosis. The antigen-antibody complexes activate the complement system and lead to the generation of "anaphylatoxins" C3a, C4a, and C5a, which can degranulate mast cells.¹⁷ Demirci *et al.*¹⁸ established that tissue parasites were important causative agents for urticaria. As a whole, it may be possible that susceptible subjects produce excessive serum total IgE levels when stimulated by *Toxocara* infection. These responses may facilitate allergic urticaria or other IgE-mediated diseases.¹⁹ Our study has shown that there was a significantly greater proportion of subjects with IgE antibodies against *Toxocara* in the allergic rhinitis group compared with the control group ($p = 0.0166$; Table 2). This can be explained by an inflammatory response stimulated by *Toxocara* infection, which may facilitate allergic rhinitis.

In the literature, this is the first clinical study to speculate that toxocariasis is a factor for allergic rhinitis. Our results indicate that there was a possible causal relationship between *Toxocara* infection and allergic rhinitis. Investigation of toxocariasis may contribute to the etiopathogenesis of allergic rhinitis. Therefore, it is our opinion that patients with allergic rhinitis from a region with a high prevalence for this parasitic infection should be evaluated for *Toxocara* infection and treatment of parasitic infection in these patients may lead to decreased symptoms.

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