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Original article

CARRIERSHIP OF ANTI-TOXOCARA IGG ANTIBODIES AMONG PATIENTS WITH CLINICAL ALLERGY

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ABSTRACT

Introduction: Toxocariasis is a zoonotic helminthosis caused by nematodes of the *Toxocara* genus. Humans are accidental hosts for the parasite. As *Toxocara* larvae migrate throughout the organism, they lead to the formation of allergic reactions.

Purpose: Our goal is to study the connection between clinical allergy and toxocariasis by examining the frequency of infection with *Toxocara spp.* and the levels of anti-*Toxocara* IgG antibodies in patients with acute urticaria, chronic urticaria and other forms of allergic reactions.

Materials and methods: Between 2018 and 2023, we tested 310 allergic patients serologically for anti-Toxocara IgG antibodies via the ELISA method. In addition we also tested 47 non-allergic people as a control group.

Results: In our study, we detected anti-Toxocara IgG antibodies in 25,48% of allergic patients. We found that patients with allergy were statistically more likely to be seropositive for anti-*Toxocara* antibodies compared to the control group. We did not find any significant difference in the levels of antibodies between different types of allergic reactions.

Conclusion: Our results show the need for serological testing for toxocariasis in all allergic patients. Additional methods and/or diagnostic markers are needed for proper assessment of cases and whether treatment is necessary.

Keywords: *Toxocara*, Toxocariasis, allergy, urticaria, ELISA,

INTRODUCTION

Toxocariasis is a zoonotic helminthosis spread by dogs and cats. It is caused by the nematodes *Toxocara canis* and *Toxocara cati* [1, 2]. Humans are accidental intermediate hosts for the parasite that get infected through the ingestion of *Toxocara* eggs, after which the larvae freed from the eggs migrate throughout the body, causing various symptoms (febrility, coughing, asthma-like attacks, hepatomegaly, CNS symptoms, allergy and eosinophilia). Because of this, toxocariasis is considered a polysystemic disease. The disease can be acute or chronic and presents as one of several possible forms - visceral, ocular, toxocariasis of the CNS and toxocariasis with other localizations (skin, muscles, myocardium) [3]. In recent years, clinical tests have shown that in cases of human toxocariasis, the host organism undergoes intoxication and sensibilization [4, 5]. As the parasite migrates, it releases excretory-secretory antigens, which are considered to be the cause of allergic reactions [6].

Our goal is to study the connection between toxocariasis and clinical allergy by examining the frequency of *Toxocara spp.* infection and the levels of anti-*Toxocara* IgG antibodies in patients with acute urticaria, chronic urticaria and other forms of allergic reactions.

MATERIALS AND METHODS

In our study, 310 patients with clinical allergy were tested for anti-Toxocara IgG antibodies in the Parasitological laboratory of the Diagnostic-Consultative Center 2, Pleven, during the period 2018 - 2023. Patients were tested based on clinical indications and were referred to the laboratory from allergological or dermatological clinics in Pleven. In order to avoid potential cross reactivity, in addition to the serological testing for anti-Toxocara IgG antibodies, all patients who were part of the study were tested serologically for echinococcosis, as well as for intestinal protozoa or helminths.

Out of the tested 310 patients, 47 (15,16%) were children or adolescents between the ages of 1-19, while 263 (84,84%) were between the ages of 20 and 86. The mean age was 44,26 (SD ± 20,90). 175 of the patients were female (56,45 %), and 135 (43,55 %) were male.

The patients were divided into three groups based on the type of allergic reaction they had, as determined by a specialist allergologist. The groups are as follows: patients with acute spontaneous urticaria - n=57 (18,39%); patients with chronic spontaneous urticaria - n=180 (58,06%); patients with other allergic reactions - n=73 (23,55%).

The urticaria cases were determined based on the criteria of the international EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria *[7]*. In the group of patients with other allergic reactions, we included patients with angioedema, asthma and allergic rhinitis.

For comparative analysis, we also included a group of 47 people without allergic symptoms. They were similarly tested in the same way as the allergic patients. The control group includes 24 females (51,06%) and 23 males (48,94%). The mean age of the control group was 54,11 (SD ± 19,62).

We examined serum samples in order to determine the presence of anti-Toxocara IgG antibodies. Venous blood for serum separation was obtained in the early morning prior to feeding in a closed system without an anticoagulant in compliance with the specified requirements for obtaining biologi-

cal material. After this, the blood underwent centrifugation to separate the serum. After which the blood underwent centrifugation to separate the serum. Haemolyzed, coagulated, lipemic blood was not used for the purposes of the study. The separated sera were each stored in 2 or more closed, chemically pure test tubes at a temperature of - 20^0 C. The frozen sera were thawed once on the day of the serological testing.

We tested patients with clinical allergy and the control group for anti-Toxocara IgG antibodies using the ELISA method. For this, we used the commercial Ridascreen Toxocara IgG kit by the manufacturer R-Biopharm AG, Germany.

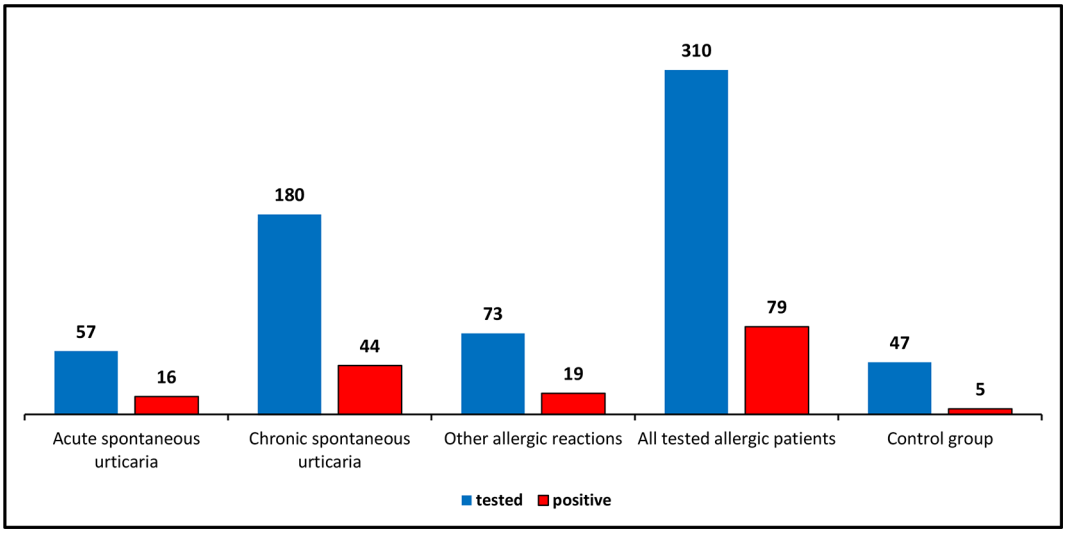
The samples were then measured with a spectrophotometer at a wavelength of 450 nm. The result was expressed as a sample ratio (SR) by dividing the optical density of each sample by the mean optical density of the two cut-offs and then adding 0,150. Samples with an SR of 0,9 or lower were considered negative, those with an SR between 0,9 and 1,1 were considered borderline results (gray zone) and samples with an SR above 1,1 were considered positive results.

Data from the study was processed using IBM SPSS Statistics 19.0 and MS Excel v. 2010. In order to determine the reliability of the results and to establish the statistical relationships between the studied phenomena, the Student-Fisher's t-test and the chi square test were used. All relationships, dependencies and differences between the studied groups are established at the level of significance of the null hypothesis - $p<0.05$.

RESULTS

Out of the 310 people with clinical allergy tested serologically, 79 (25,48%) were positive for anti-Toxocara IgG antibodies. Out of the 47 people in the control group, 5 (10,63%) were carriers of anti-Toxocara antibodies. Fig. 1 shows the distribution of positive cases among tested patients based on the type of allergic reaction, as well as in the control group.

Fig. 1. Distribution of positive antitoxocara IgG antibody results among different groups of allergic reactions and in the control group.

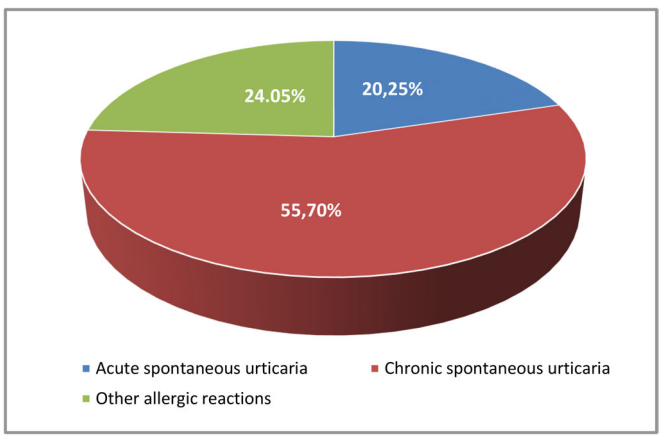


In our study, there was a statistically significant difference between the carriership of anti-*Toxocara* IgG antibodies in patients with clinically manifested allergy and the control group (χ^2 = 4,999; p=0,025). A similar statistical difference was also found when comparing the frequency of positive cases among patients with acute spontaneous urticaria compared to the control group (χ^2 = 5,063; p = 0,024); between patients with chronic spontaneous urticaria and the control group (χ^2 = 4,133; p = 0,042); and between patients with other allergic reactions and the control group (χ^2 = 4,232; p = 0,040).

In our data set, 55,70% of the positive results were from patients with chronic spontaneous urticaria compared to the positive cases among patients with acute spontaneous urticaria cases and among those with other allergic reactions, which make up 20,25% and 24,05% of the total number of positive patients respectively. This difference in distribution between the three groups is presumably due to the different numbers of examined patients in each group.

Fig. 2 shows the relative share of positive cases for anti-*Toxocara* antibodies in each of the three groups of allergic reactions.

Fig. 2. Relative share of positive patients for anti-*Toxocara* IgG antibodies based on the type of allergic reaction



A comparison between the the frequency of positive serological results and the mean level of anti-Toxocara IgG antibodies in each group of allergic patients is shown in Table 1:

Table 1. Frequency and mean level of anti-*Toxocara* IgG antibodies based on type of allergic reaction

Diagnosis	Positive cases for anti-*Toxocara* IgG antibodies		Antibody level (SR)		Mean level of antibodies (SR)
	n	%	Minimum	Maximum	
Acute spontaneous urticaria	16	28,07	1.2	3.4	2.0025 SD ± 0,781
Chronic spontaneous urticaria	44	24,44	1.2	6.8	2.3747 SD ± 1,464
Other allergic reactions	19	26,03	1.3	7	2.6731 SD ± 1,510

28,07% of the patients of acute spontaneous urticaria were positive for anti-Toxocara antibodies, compared to 24,44% in patients with chronic spontaneous urticaria and 26,03% in patients with other allergic reactions. This result was not statistically significant (χ^2 = 0,424; p = 0,809).

We also did not find any significant difference between the levels of antibodies between patients with acute and chronic urticaria (p=0.339), between those with acute urticaria and other allergic reactions (p=0.119), nor between patients with chronic urticaria and other allergic reactions (p=0.465).

DISCUSSION

One of the main pathogenetic mechanisms in helminthic diseases is the toxo-allergic effect due to the complex immunopathological reactions in the organism of the host. Skin allergies are one of the first symptoms of parasitoses, and their persistence often affect the quality of life of these patients [8].

The complex migration of *Toxocara spp.* in the human host's body, the long persistence of anti-*Toxocara* IgG antibodies and the frequency of cross reactions and false positive results creates diagnostic and therapeutic difficulties in daily clinical practice.

In this study, we investigated the frequency of anti-*Toxocara* IgG antibody carriership in patients with different types of allergic reactions. Roughly one-quarter (25,48%) of the examined patients were positive for anti-*Toxocara* IgG antibodies. Similarly high percentages of positive serological results for anti-Toxocara IgG antibodies have been reported by various other researchers: seropositivity of 16% among children with urticaria in Brazil [9]; 31,8% of 753 patients with allergic symptoms in a study that took place in Italy [10] and 38,1% of children with chronic pruritus in France [11]. In Bulgaria the first clinic-diagnostic studies on toxocariasis were done by Rainova I, and Kaneva E, et al. [12, 13]. Serological testing of 386 people that passed through the parasitological laboratory of the National Center for Infectious and Parasitic diseases in Sofia over a three-year period showed that 22,3% of the tested people were positive for anti-Toxocara IgG antibodies, and 17,6% of the positive ones had allergic symptoms [14].

Similarly to other case-control studies, we found that there was a statistically significant difference between the frequency of anti-Toxocara antibody carriership in allergic patients and the control group of non-allergic people (p=0,025) [9, 11, 15, 16]. Most authors consider that the connection between toxocariasis and allergy is not coincidental, however, it should be noted that some studies have not found a connection between the two [13, 14, 17].

Our study raises a few questions, the most important of which is whether being infected by *Toxocara spp.* causes the development of the encountered allergic symptoms. Allergic diseases are multifactor diseases that require both genetic factors and factors from the external environment to develop [18]. Parasitic diseases may act as a co-factor in the pathogenesis of allergic reactions in humans. Proper diagnosis for toxocariasis requires the detection of anti-*Toxocara* IgG antibodies and the determination of the stage of the invasion in order to elucidate whether treatment is necessary in the specific case.

Humans are an accidental host for *Toxocara spp.,* and because of this, the parasite does not reach sexual maturity inside a human host and does not release invasive elements (eggs) in the stool. The diagnosis is based on the indirect effect of the parasite – testing for the presence of anti-*Toxocara* IgG antibodies in a serum sample from the patient. The main diagnostic methods used are ELISA and Western blot. In routine practice, ELISA is most often used for diagnosis. Serological diagnosis of toxocariasis does have its own faults. One of which is the possibility for false positives due to cross reactions between the excretory-secretory antigens of *Toxocara spp.* and the antigens of other helminths (*Trichinella, Ascaris.*

Strongyloides) [19]. The presence of anti-*Toxocara* IgG antibodies only shows that the person has had contact with the parasite, however, it cannot determine the amount of time passed since infection, nor can it determine the type of infectious process in question – acute, chronic, latent or merely persistence of antibodies. It is known that anti-*Toxocara* IgG antibodies detected with ELISA can persist for up to 2,7 years, while the same antibodies detected with Western blot can persist for up to 5 years [20]. This creates difficulties in the correct assessment of cases. In this line of thinking, the physician's behavior towards patients with allergic reactions must be considered carefully.

CONCLUSION

The high frequency of antitoxocara IgG antibodies detected among patients with different types of allergic reactions in both our and other studies necessitates the serological examination for toxocariasis of allergic patients in general. In order to determine the stage of infection and the necessity of treatment, other additional clinical, serological and laboratory criteria are needed. In the development and routine application process, methods are used to determine the avidity of anti-*Toxocara* IgG antibodies and the detection of early IgA antitoxocara antibodies. The levels of total IgE and/or the levels of eosionophil cationic protein could be used as additional lab markers in patients infected with *Toxocara spp.* Additional studies are required to determine how real the link between allergy and toxocariasis is and what course of action the physician should take in these cases.

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