

***Blastocystis* sp. and *Toxocara* spp. coinfection in Patients with Clinically Manifested Skin Allergy**

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Abstract

In recent years, more and more publications reflect the relationship between clinically apparent allergy and infection with *Blastocystis* spp. or *Toxocara* spp. Our main goal is to examine allergy patients infected with *Blastocystis* sp. for the presence of anti-*Toxocara* IgG antibodies, intending to detect co-infection and its influence on the allergic condition of patients. For a period of 3 years (2020 – 2022), 1011 patients hospitalized in the Allergology Clinic at the University Hospital, Pleven, were examined for intestinal protozoa and helminths. Fresh stool samples for microscopic parasitological examination were used. From persons infected with *Blastocystis* sp. venous blood was obtained for the determination of specific anti-*Toxocara* antibodies. Of the patients with allergic symptoms examined for intestinal helminths and protozoa, 40 were infected with *Blastocystis* sp. Out of them, seven (17.5%) were positive for anti-*Toxocara* IgG antibodies on the ELISA test, and Western blot confirms the diagnosis. All individuals with *Blastocystis* / *Toxocara* co-infection were established against the background of chronic urticaria, but no difference was found in the severity of clinical manifestations in comparison with those with only blastocystosis and those without evidence of parasitic infection. The data of our study, give us reason to consider that the establishment of parasitic infections in persons with skin allergic manifestations is not unusual, but in general, it is very difficult to determine whether they are the cause of the allergic condition or are a finding in the course of diagnostic clarification.

Keywords: *Blastocystis* sp.; *Toxocara* sp.; co-infection; chronic urticaria

Резюме

През последните години все повече публикации отразяват връзката между клинично изявената алергия и инфекцията с *Blastocystis* spp. или *Toxocara* spp. Нашата основна цел бе да изследваме пациенти с алергични прояви, заразени с *Blastocystis* sp. за наличие на анти-*Toxocara* IgG антитела, с оглед откриване на съпътстваща инфекция и нейното влияние върху алергичното състояние на пациентите. За период от 3 години (2020 – 2022 г.) са изследвани за чревни протозои и хелминти 1011 пациенти, хоспитализирани в Клиниката по алергология към УМБАЛ – Плевен. Използвани са пресни фекални проби за микроскопско паразитологично изследване. От лицата, диагностицирани с *Blastocystis* sp. е взета венозна кръв за определяне на специфични анти-*Toxocara* антитела. От пациентите с алергични симптоми, изследвани за чревни хелминти и протозои, 40 бяха заразени с *Blastocystis* sp. От тях седем (17.5%) се установи наличие на анти-*Toxocara* IgG антитела в ELISA теста, а чрез Western blot диагнозата бе потвърдена. Всички лица с *Blastocystis* / *Toxocara* коинфекция бяха установени на фона на хронична уртикария, но не се установи разлика в тежестта на клиничните прояви в сравнение с тези само с бластоцистоза и тези без данни за паразитна инфекция. Данните от нашето изследване ни дават основание да считаме, че установяването на паразитни инфекции при лица с кожни алергични прояви не е необичайно, но като цяло е много трудно да се определи дали те са причина за алергичното състояние или са случайна находка в хода на диагностичното уточняване.

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Introduction

Allergic reactions are among the most common conditions in modern times (Murrison *et al.*, 2019). Genetically determined predispositions, as well as external and internal triggering factors, are important for the development of atopy (Burbank *et al.*, 2017; Murrison *et al.*, 2019). Despite the lack of consensus on the relationship between parasitic and allergic diseases, today many authors consider that with their pathological mechanisms on the host organism, parasites induce the development of endogenous intoxication, sensitization, autoimmune reactions, and modulation of the immune response (Cooper, 2009; Minciullo *et al.*, 2018; Fernandes *et al.*, 2019; Maizels, 2020). Allergic reactions have been associated with infection from both intestinal and tissue parasites (Kolchir *et al.*, 2016). In recent years, more and more publications reflect the relationship between clinically apparent allergy and infection with *Blastocystis* spp. or *Toxocara* spp. (Matos *et al.*, 2017; Bahrami, 2020).

Blastocystis spp. is a unicellular microorganism widely isolated from humans and animals, which is spread via the fecal-oral route, through contaminated factors of the external environment with either human or animal feces. The genus *Blastocystis* contains 17 subtypes (STs) of which nine have been isolated from people (Stensvold, 2013; Skotarczak, 2018). Blastocystosis can present asymptotically or with abdominal pain and diarrhea, however, *Blastocystis* sp. has also been isolated from patients with allergic symptoms (Kantardjiev *et al.*, 2019; Bahrami, 2020).

Toxocariasis is a zoonotic parasitic infection, caused by the migration of larvae of the helminths *Toxocara canis* and *T. cati* to various internal organs of the infected person such as the liver, lungs, skeletal muscles, heart, brain, and eyes. The clinical presentation of toxocariasis depends on the location of the parasite in the host and is often accompanied by allergic symptoms such as - acute or chronic urticaria, angioedema, and bronchial asthma (Rostami *et al.*, 2019).

The study of the disease process in mixed parasitic infections with common routes of transmission is of interest to specialized medical science. It is assumed that the co-infection aggravates the parasitic disease process and affects the severity and the variety of symptoms of clinically apparent allergy. Our goal is to examine patients with clinically presented allergy conditions, and infected with *Blastocystis* sp. for the presence of anti-*Toxocara* IgG antibodies, to detect co-infection and its

influence on the allergic condition of patients.

Patients and Methods

Examined Patients

For a period of 3 years (2020–2022), 1011 patients hospitalized in the Allergology Clinic at the University Hospital – Pleven, were examined for intestinal protozoa and helminths. Forty patients with blastocystosis were tested for the presence of anti-*Toxocara* IgG antibodies in the National Centre of Infectious and Parasitic Diseases, Sofia. Of them, 14 were male (35%) and 26 were female (65%). The age of the patients varied between 14 and 76 years (mean age 49.63, SD±16.12). In terms of symptoms, 26 (65%) of the patients were with chronic spontaneous urticaria, 12 (30%) with acute spontaneous urticaria, and two (5%) had angioedema.

Samples for parasitological examinations

Fresh fecal samples for microscopic parasitological examination were used. They were brought to the laboratory in sterile and chemically pure vessels. From persons infected with *Blastocystis* sp. venous blood was obtained in a closed system. For the determination of specific anti-*Toxocara* antibodies, separated serum from the blood samples was used.

Diagnostic methods

The blastocystosis diagnosis was determined via light microscopy of wet mounts stained with iodine solution. Our main criterion for the diagnosis was the presence of the vacuolar form of the parasite in the wet mount. Diagnosis of toxocariasis was performed via the serological method ELISA with an ELISA *Toxocara* IgG kit (R-Biopharm, Germany), according to the manufacturer's instructions. The results were expressed as a Sample Index (SI) and were calculated by dividing the extinction value of the sample by the value of the cut-off. Samples with an SI<0.9 were considered to be negative, ones with an SI between 0.9 and 1.1 were borderline, and samples with an SI above 1.1 were positive. All samples positive or borderline on the ELISA test were examined via a Western blot IgG test (LD BIO, France) for confirmation. The appearance of two or more low molecular weight bands between 24 and 35 kDa indicates the presence of specific anti-*Toxocara* IgG antibodies in the patient sample.

Statistical analysis

Statistical data processing was performed using the statistical software SPSS version 16.0.

Some statistical quantities such as Mean were defined, and the ANOVA test was used to determine if there were statistically significant differences. Values of $p \leq 0.05$ were accepted as statistically significant.

Ethical considerations

The study was reviewed and approved by the institutional review board at the Medical University - Pleven and informed consent was obtained from the patients.

Results

From the total 1011 patients with allergic symptoms examined for intestinal helminths and protozoa, 40 were infected with *Blastocystis sp.* The prevalence of this protozoan parasite among the studied allergic individuals was 3.95%. The distribution by sex, age, and allergic condition of the 40 patients with blastocystosis examined for anti-*Toxocara* antibodies is presented in Tables 1 and 2. The most affected groups among the examined persons were females (65%) and people of active working age (15-44) - 37.5%. Most of the patients with blastocystosis had chronic spontaneous urticaria (65%).

Out of the 40 tested people with blastocystosis and clinically manifested allergy, 7 (17.5%) were positive for anti-*Toxocara* IgG antibodies on the ELISA test. Out of them, five were female (19.23% out of the 26 tested women) and two were male (14.26% out of the 14 tested men). The SI of the positive patients varied between 1.21 and 7.82 (mean value of 3.01). The mean value of the SI

was higher in women (3.63) than in the tested men (1.48), although statistical processing showed no statistically significant differences ($p=0.318$). The highest number of positive patients is in the 60-74 age group (3 out of 10), followed by the 45-59 age group (3 out of 11) and a single positive result is in the 15-44 age group. The highest SI values are in patients above 60 years of age - 3.99, but the statistical analysis did not show any association between age and seropositivity for toxocarasis ($p=0.687$).

In terms of the type of allergic reactions, all patients positive for anti-*Toxocara* IgG antibodies had chronic spontaneous urticaria (Table 3).

In all seven of the ELISA *Toxocara* IgG-positive patients, Western blot showed the characteristic bands, which confirms the diagnosis of toxocarasis in persons with allergic manifestations and blastocystosis (Fig. 1).

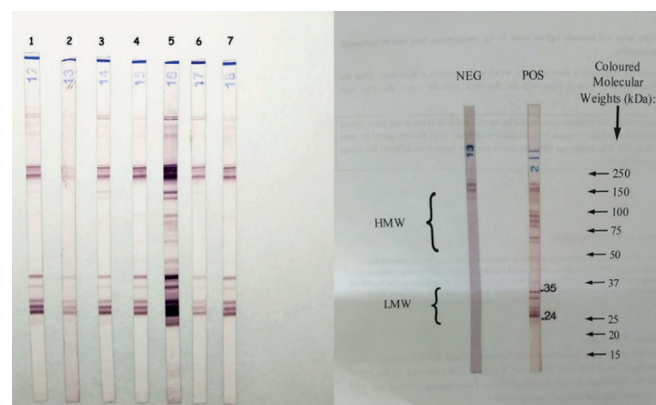


Fig. 1. Results of Western blot in the patients positive for anti-*Toxocara* IgG antibodies on the ELISA test

Table 1. Distribution of patients infected with *Blastocystis sp.* by sex and type of allergic reaction

Type of allergic reaction	Sex				Total	
	males		females		N	%
	N	%	N	%		
Acute spontaneous urticaria	3	7.5	9	22.5	12	30
Chronic spontaneous urticaria	11	27.5	15	37.5	26	65
Angioedema	-	-	2	5.0	2	5
Total	14	35.0	26	65.0	40	100

Table 2. Distribution of patients infected with *Blastocystis sp.* by age and type of allergic condition

Type of allergic condition	Age groups										Total	
	0-14		15-44		45-59		60-74		75-89		n	%
	n	%	n	%	n	%	n	%	n	%		
Acute spontaneous urticaria	-	-	5	12.5	2	5	4	10	1	2.5	12	30
Chronic spontaneous urticaria	1	2.5	8	20	11	27.5	6	15	-	-	26	65
Angioedema	-	-	2	5	-	-	-	-	-	-	2	10
Total	1	2.5	15	37.5	13	32.5	10	25	1	2.5	40	100

Table 3. Characteristics of patients infected with *Blastocystis* sp. and seropositive for toxocarosis

No	Sex	Age	Type of allergic condition	ELISA (SI)	WB
1.	f	52	chronic spontaneous urticaria	1.97	(+)
2.	m	53	chronic spontaneous urticaria	1.21	(+)
3.	m	70	chronic spontaneous urticaria	1.74	(+)
4.	f	67	chronic spontaneous urticaria	2.41	(+)
5.	f	70	chronic spontaneous urticaria	7.82	(+)
6.	f	39	chronic spontaneous urticaria	1.54	(+)
7.	f	55	chronic spontaneous urticaria	4.4	(+)

Discussion

This study is a first for the country regarding the role of *Blastocystis* / *Toxocara* co-infection on the etiology and clinical manifestation of allergic conditions in humans. According to the literature, infections with intestinal and tissue parasites are associated with clinically manifested allergic conditions, but a clear causal link between them has not been established to date (Maizels, 2013; Arik Yilmaz *et al.*, 2016). In this regard, we decided to investigate whether characteristic allergic manifestations exist and what their severity is in coinfection with two parasite species that are often associated with clinically manifested allergy, namely *Blastocystis* spp. and *Toxocara* spp.

Despite the debatable role of *Blastocystis* sp. as a human pathogen, there is evidence of a connection between blastocystosis and the development of acute or chronic spontaneous urticaria (Bahrami *et al.*, 2020). According to a study in Egypt, *Blastocystis* sp. is found in 43.3% of people with bronchial asthma 15.4% of people with urticaria and asthma, and 8.6% of people with urticaria (El Saftawy *et al.*, 2019). The pathogenesis of the cutaneous symptoms in blastocystosis is unclear. Some authors consider a possible link between the symptoms and the subtype of the parasite. Out of the nine *Blastocystis* sp. subtypes isolated from people, ST3 as well as ST1 and ST2 are the most commonly isolated ones from patients with chronic urticaria (Roberts *et al.*, 2014; Bahrami *et al.*, 2020; Aykur *et al.*, 2022). It is considered that the density of the infection and interactions between the intestinal microbial communities are responsible for the allergizing role of *Blastocystis* (Billy *et al.*, 2021; Deng *et al.*, 2022).

Toxocara spp. is a parasite that can evade the host's immune response, which explains the chronification of the invasion and the persistence of antibodies (Maizels, 2013). Toxocarosis is characterized by diverse and non-specific symptoms and often remains undiagnosed. Various allergic

responses such as urticaria, prurigo, eczema, and angioedema have been linked to *Toxocara* seropositivity (Gavignet *et al.*, 2008; Burak Selek *et al.*, 2015; Mohammadzadeh *et al.*, 2018; Temsah *et al.*, 2021). Interactions between different parasitic species can be synergistic or antagonistic, which can affect the severity of both the parasitic infection and the allergic disease (Blackwell *et al.*, 2013).

The data from our study shows that all individuals with *Blastocystis* / *Toxocara* co-infection were established against the background of chronic urticaria, and in our opinion, it is more likely a parasitic infection in the course of chronic urticaria, but we could not categorically exclude the possibility of chronic urticaria, as a consequence of a parasitic infection. According to some authors (Kolkhir *et al.*, 2016), parasitic infections are an uncommon underlying cause of chronic urticaria but are relatively common in preexisting urticaria (range: 0–75.4%) (Kolkhir *et al.*, 2016). According to the same authors, patients with chronic spontaneous urticaria had more frequent infections with *Blastocystis hominis* and had a significantly higher risk of seropositivity for *Toxocara canis* compared with healthy controls. In this respect, the data from our study are similar to those in the literature. Regarding the distribution of patients with blastocystosis by age and sex, there is a predominant involvement of persons over 14 years of age (over 97%) and of the female gender (65%). While the distribution by age could be explained by the fact that the children's contingents are hospitalized and treated in specialized children's clinics/wards, the distribution by gender is difficult to explain because the female gender has better hygiene habits. In the co-infected persons, the distribution by age and gender was identical. All individuals are adults and females predominate. No difference was found in the severity of clinical manifestations in those with chronic urticaria and *Blastocystis* / *Toxocara* co-infection, those with blastocystosis, and those without evidence of parasitic infection. This gives

us reason to believe that the specific *Blastocystis* / *Toxocara* co-infection does not lead to more serious manifestations aggravating the allergic condition.

Conclusion

The data of our study, although it is regional, give us reason to consider that the establishment of parasitic infections in persons with skin allergic manifestations is not unusual, but in general, it is very difficult to determine whether they are the cause of the allergic condition or are a finding in the course of diagnostic clarification because in most cases the etiological treatment does not lead to a complete remission of the allergic condition.

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