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Original Article

Serum Eosinophil Cationic Protein in Urticaria Patients with Anti-*Toxocara* IgG Antibodies

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Abstract

Background: Toxocariasis is a parasitic disease that affects both humans and animals and is caused by migration of helminth larvae of *Toxocara spp.* in the host. It often presents with allergization such as urticaria, asthma-like symptoms and/or eosinophilia. Standard diagnosis is via the discovery of specific anti-*Toxocara* IgG antibodies which are difficult to interpret, which is why additional diagnostic criteria are necessary. We aimed to determine the levels of eosinophil cationic protein in patients with acute and chronic spontaneous urticaria with or without anti-*Toxocara* IgG antibodies, in order to assess the value of eosinophil cationic protein (ECP) for the diagnosis of covert cases of toxocariasis among patients with clinical allergy.

Methods: We examined ECP levels in 48 patients with urticaria who were *Toxocara*-IgG positive, in 45 patients with urticaria with a negative result for anti-*Toxocara* IgG and in 50 healthy controls without allergic symptoms or anti-*Toxocara* antibodies.

Results: Median serum ECP levels were significantly higher in patients with urticaria compared to the controls (P=0.007). We also determined that median ECP levels were significantly higher in patients with acute urticaria that were carriers of anti-*Toxocara* antibodies, compared to acute urticaria patients without anti-*Toxocara* antibodies (P=0.040). There was a significant positive correlation between ECP and anti-*Toxocara* IgG antibody levels (P = 0.024).

Conclusion: ECP could be used as an additional marker to assess cases of potential "latent" toxocariasis among urticaria patients.

Introduction

Toxocariasis is a widely distributed parasitic disease that affects both animals and people. It is caused by helminths of the *Toxocara* genus – *T. canis* and *T. cati* (1). The clinical manifestations result from the continuous migration of the parasite's larvae within the host and may range from asymptomatic carriage or subclinical presentation to visceral, ocular or neurological forms of the disease (2). Allergization of the host is common and often presents with urticaria, asthma,

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pruritus and/or eosinophilia. The allergic symptoms are caused by the secretion of excretory-secretory (ES) antigens released from the migrating larvae (3, 4). The frequency of urticaria among patients with toxocariasis varies in the literature. A meta-study on the association between nematode infections and urticaria found that between 14,5% and 29% patients with urticaria were positive for anti-*Toxocara* IgG antibodies, while in a study among children in Brazil with anti-*Toxocara* IgG antibodies, 46,5% of them had urticaria (5,6).

Diagnosis of toxocariasis is complex. It includes epidemiological data, lab tests and is confirmed via the discovery of anti-*Toxocara* IgG antibodies in the serum of the patient. Due to the persistence of these antibodies and the possibility of cross reactions, results are often difficult to interpret (7). To improve diagnosis of toxocariasis, in addition to clinical data and serological results, new criteria are necessary to determine to stage of the invasion, and whether etiological treatment is necessary.

Eosinophils are a fraction of granulocytes which are active in the pathogenesis of both allergic and parasitic diseases (8-10). During helminth invasions there is an increased secretion of Th2 - associated cytokines which leads to peripheral eosinophilia and local tissue accumulation of eosinophils around the invading parasites (9). As a part of the local antiparasitic immune response, eosinophils secrete from their granules a group of cytotoxic proteins, one of which is eosinophil cationic protein (ECP). ECP is a member of the ribonuclease family and it has been demonstrated in vitro that in high concentrations it can paralyze or kill migratory parasites in the tissues of the host (11). It has been found that ECP levels are an eosinophil mediated marker for the intensity of invasion with the helminth Ancylostoma duodenale and for the severity of ancylostomiasis in humans (9).

We aimed to determine and compare serum ECP levels in patients with acute and chronic spontaneous urticaria who are carriers of anti-*Toxocara* IgG antibodies, and to evaluate the potential value of this biomarker in diagnosing "covert" cases of *Toxocara* infection among patients with clinical allergy.

Materials & Methods

The study is part of project funded by Medical University – Pleven approved by the university's ethics committee (Protocol № 72/№ 749 – KENID/05.06.2023). Written informed consent was obtained from the subjects that are part of the study via standardized form.

We studied the levels of eosinophil cationic protein among 93 patients with acute urticaria (AU) and chronic spontaneous urticaria (CSU). Out of the 93 tested, 48 had a positive serological result for anti-*Toxocara* IgG antibodies. We also measured ECP levels in a control group consisting of 50 individuals with no prior history of allergic symptoms and with a negative result for anti-*Toxocara* IgG antibodies. Subjects with intestinal or tissue parasites were not included in any of the cohorts.

The median age of the patients with urticaria and anti-*Toxocara* IgG antibodies was 64 years (range: 21 – 76 years old). Depending on the type of urticaria 21 of them had AU (43.8%) and 27 had CSU (56.3%). In the group, 12 were male (25%) and 36 were female (75%). In the group of patients with urticaria with a negative serological result for anti-*Toxocara* antibodies, 18 had acute urticaria (40%) and 27 had chronic spontaneous urticaria (60%). The median age is 53 years (range: 12 – 78 years old), while 15 of the group were male (33.3%) and 30 were female (66.7%). The median age of the individuals in the control group was 57.50 (min - 9; max - 86). Twenty-three of them were male (46%) and 27 were female (54%). The type of urticaria was determined based on the criteria of the international EAACI /GA²LEN /EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria (12). A persistent urticarial rash with no clear cause and duration less than 6 weeks is considered acute urticaria according to the guideline, while urticaria of the same type that lasts more than 6 weeks is considered chronic spontaneous urticaria. In order to determine the levels of ECP and anti-

Toxocara IgG antibodies we used serum liberated from venous blood samples obtained early in the morning before breakfast. Serum samples were stored at - 80^0 C until the day of the serological testing.

Anti-*Toxocara* IgG antibodies were detected using the ELISA method with the Ridascreen *Toxocara* IgG kit (R-Biopharm AG Germany; Sensitivity – 100%; Specificity – 90,7%), based on the instructions of the manufacturer. We used a spectrophotometer with a light frequency of 450 nm to determine the presence of antibodies in each sample. The results are expressed as a Sample Ratio (SR), which is calculated by dividing the optical density of each sample via the sum of the optical density of the two cut-off controls and 0,150. Positive results are those with an SR above 1,1, while results with an SR under 0,9 are considered negative. Sample ratio between 0,9 and 1,1 is considered an intermediate result that requires retesting.

We measured ECP levels with the kit Human Eosinophil Cationic Protein (ECP) ELISA kit (CUSABIO - China), following the instructions of the manufacturer. In order to calculate the ECP levels a calibration curve is constructed by using a series of diluted standards with the following fixed concentrations: S7 - 100 ng/mL; S6 - 50 ng/mL; S5 - 25 ng/mL; S4 - 12,5 ng/mL; S3 - 6,25 ng/mL; S2 - 3,12 ng/mL; S1 - 1,56 ng/mL; S0 - 0 ng/mL. The optical density of the samples and standards was measured at a wave length of 450 nm. The kit's minimal detection limit for eosinophil cationic protein is 1,56 ng/mL.

Statistical analyses were performed using SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA) and Microsoft Excel. Pearson correlation and Kruskal–Wallis tests were applied to assess differences between groups, with statistical significance set at $P \leq 0.05$.

Results

Table 1 presents the serum ECP levels in the three study groups.

Among patients with AU and CSU that were carriers of anti-*Toxocara* IgG antibodies, the levels of eosinophil cationic protein varied between 11.21 ng/mL and 121.31 ng/mL (median - 36.77 ng/mL). In the group of urticaria patients without anti-*Toxocara* antibodies, ECP levels were between 1.56 ng/mL and 119.71 ng/mL (median - 28.48 ng/mL). ECP values in the control group varied between 1.56 ng/mL and 62.27 ng/mL (median - 25.48 ng/mL).

Table 1: Levels of ECP (ng/ml) among the studied subjects based on the type of allergic reaction

Group	Diagnosis	ECP level (ng/ml)		Median levels of ECP (ng/ml)
		Minimum value	Maximum value	
Patients with urticaria, positive for anti-*Toxocara* IgG antibodies (n=48)	Acute urticaria (n= 21)	11.21	90.80	39.51
	Chronic spontaneous urticaria (n=27)	13.35	121.31	36.60
	Total	11.21	121.31	36.78
Patients with urticaria, negative for anti-*Toxocara* IgG antibodies (n=45)	Acute urticaria (n=18)	1.56	43.37	28.96
	Chronic spontaneous urticaria (n=27)	1.56	119.71	28.48
	Total	1.56	119.71	28.48
Healthy control group (n=50)		1.56	62.27	25.48

Among patients with AU that were carriers of anti-*Toxocara* IgG antibodies, the median level of ECP is 39.51 ng/mL, while among those with AU with no anti-*Toxocara* antibodies it was 28.96 ng/mL. Median ECP levels in subject with CSU with and without anti-*Toxocara*

antibodies were 36.60 ng/mL and 28.48 ng/mL respectively.

We found statistically significant higher median value of ECP in patients with urticaria and a positive result for anti-*Toxocara* IgG antibodies and in patients with urticaria with no anti-*Toxocara* IgG antibodies compared to the healthy controls (H=9.867, df=2, *P*=0.007) (Fig. 1).

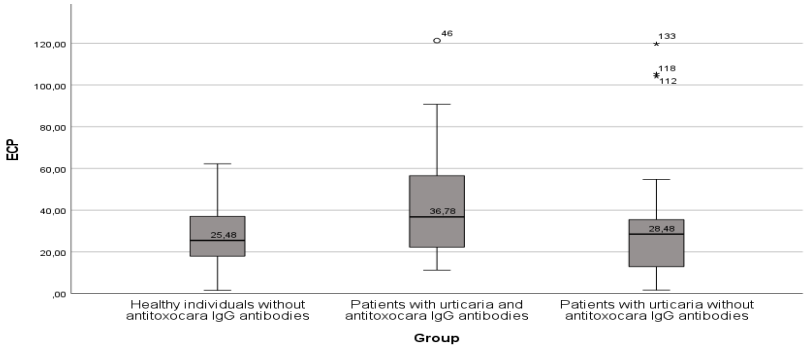

Fig. 1: Comparison of ECP (ng/ml) values in the three studied groups

Additionally, we also found a significant difference in the levels of ECP between the two urticaria groups - with and without the presence of anti-*Toxocara* IgG antibodies (H=6.841, df=1, *P*=0.009).

The levels of ECP were statistically higher in patients with acute urticaria that were carriers of anti-*Toxocara* IgG antibodies, compared to patients with acute urticaria with a negative result for anti-*Toxocara* IgG antibodies (H=4.232, df=1, *P*=0.040). We did not find a significant difference in the levels of ECP between patients with chronic spontaneous urticaria with and without anti-*Toxocara* antibodies (H=3.213, df=1, *P*=0.073). The difference in ECP levels between patients with acute and chronic urticaria is not significant (H=0.146, df=1, *P*=0.703).

We found a positive correlation between the levels of ECP and the levels of anti-*Toxocara* IgG antibodies in patients with AU (ϱ = 0.360; *P* = 0.024). As anti-*Toxocara* IgG antibodies increase there is a tendency for ECP values to also increase among patients with acute urticaria (Fig. 2).

No statistically significant correlation was observed between serum ECP levels and anti-*Toxocara* IgG antibodies in patients with chronic spontaneous urticaria (ϱ = 0.181; *P* = 0.191).

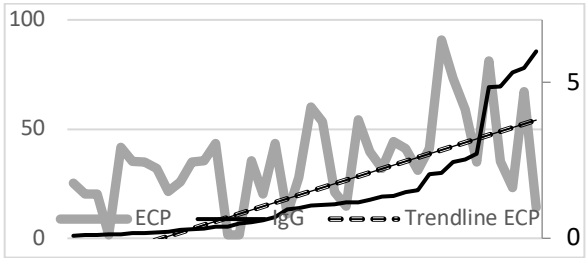

Fig. 2: Comparison between the levels of ECP (ng/ml) and anti-*Toxocara* IgG antibodies in patients with acute urticaria (Pearson correlation)

Discussion

ECP was first identified in 1977 and, despite its short half-life, its serum levels are considered to reflect the overall burden of activated eosinophils within the host organism (13). Its role in the pathogenesis of helminth diseases is not well studied, but various studies have proven its role as a part of the local immune response (11). In clinical practice it is common to encounter cases of AU or CSU in patients who also carry anti-*Toxocara* IgG antibodies. We consider that those could be cases of covert toxocariasis, the beginning of which is dif-

ficult to determine and which cannot be differentiated from the persistence of anti-*Toxocara* antibodies.

The significant difference in median ECP levels in our study between urticaria patients and healthy controls is confirmed by other studies in the available literature. Kaneva et al. found a statistically significant difference in ECP levels between patients positive for anti-*Toxocara* IgG antibodies and healthy blood donors (14). In a French study among patients with covert toxocariasis, the authors report a significant difference in mean levels of ECP between patients with allergic symptoms and patients without allergies (15).

The correlation between ECP and anti-*Toxocara* IgG levels in patients with acute urticaria described in our study is of interest, since ECP is one of the cytotoxic molecules secreted by eosinophils. This could represent a recent infection with *Toxocara* spp., which coincides with an intense local eosinophilic reaction. A similar result has been reported in the literature. Choi et al. described a significant correlation between levels of ECP and the intensity of the local eosinophilic tissue reaction in toxocariasis (16).

Also, there exist several studies on the changes of ECP in patients with toxocariasis before and after etiological treatment. Magnaval et al. described a case of an 11-year-old girl with asthma-like symptoms, a positive serological result for anti-*Toxocara* IgG antibodies and increased levels of ECP of 28 µg/L. After 21 days of treatment with the antihelminthic drug diethylcarbamazine, they found that ECP levels decreased to 8 µg/L (17). Niedworok et al. found a statistically significant reduction in ECP levels in anti-*Toxocara* IgG antibody carriers 6 months after therapy with Mebendazole (18).

We should, however, mention that changes in ECP levels can be found not just in helminthic diseases. Elevated ECP levels are also found in some atopic diseases like bronchial asthma and allergic rhinitis, in cases of eosinophilic neoplastic processes and in a few bacterial diseases such as tuberculosis (11, 19). In a study which compared the levels of serum ECP in patients with helminthic diseases and ones with atopic dermatitis, the median level of ECP among the patients with atopic dermatitis was 50 µg/L, compared to 90 µg/L in patients with hookworm disease and 98 µg/L in patients with onchocerciasis (20). In this context, our findings in patients with acute and chronic spontaneous urticaria should be interpreted with caution in diagnostic practice.

Conclusion

A positive correlation was observed between anti-*Toxocara* antibody levels and ECP levels in individuals with acute urticaria. These findings might be applied in clinical practice to aid in the evaluation of "latent" toxocariasis among patients with urticaria and to support consideration of parallel antiparasitic therapy in addition to standard antiallergic treatment.

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Conflict of Interests

The authors declare no conflict of interest.

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