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Original Article

Eosinophilia and Helminthic Infections in the Black Sea Region of Türkiye

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Abstract

Introduction: Eosinophils play crucial roles in the cellular and humoral immune responses and usually constitute 1-3% of leukocytes in peripheral blood. Conditions that cause eosinophilia include allergic diseases, drug hypersensitivity, infections, cancers, and immunological or autoimmune diseases. Among parasitic diseases, it has been reported that eosinophilia is most frequently caused by helminth infections such as cystic echinococcosis (CE), fascioliasis, toxocariasis, schistosomiasis, strongyloidiasis, filariasis, and trichinellosis.

Methods: The population of the research was taken as the population of Samsun province. A total of 646 sera were taken from patients (278 males and 279 females) with eosinophilia (>5%), and 89 samples were taken from patients (45 males and 44 females) without eosinophilia (<5%) which were referred to the Public Health Laboratory for hemogram analysis from Samsun province were included in the study. An in-house ELISA was used to investigate *F. hepatica*, *T. spiralis,* and *E. granulosus* antibodies in sera collected from the patients in the eosinophilic and non-eosinophilic groups.

Results: In the eosinophilic group (n=557), seropositivity rates were 0.9% for echinococcosis, 0.5% for fascioliasis, 72.2% for toxocariosis, and 0.5% for trichinellosis, while they were 0.0%, 0.0%, 64%, and 1.1%, respectively, in the non-eosinophilic group (89 cases).

Conclusion: Although the results suggest that helminth infections may be relatively rare causes of eosinophilia in our region, more data are required due to the low number of controls. For this purpose, a comprehensive seroprevalence study with a larger control group will investigate other helminthic agents such as ascariasis, strongyloidiasis, and hookworm infections.

Keywords: Eosinophilia, Helminthic infections, Black sea region, Türkiye

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Introduction

Eosinophils play crucial roles in the cellular and humoral immune responses and usually constitute 1-3% of leukocytes in peripheral blood. Eosinophilia is an eosinophil count of >1,500/mm^3 in peripheral blood or an eosinophil proportion exceeding 5% of all circulating leukocytes (1-3). Conditions that cause eosinophilia include allergic diseases, drug hypersensitivity, infections, cancers, and immunological or autoimmune diseases. While eosinophilia is associated with atopic diseases in developed countries, helminth infections are the most common cause of eosinophilia worldwide (1-3). Eliminating helminths is generally associated with type-2 immune responses characterized by the induction of IgE release, increased levels of eosinophils and mast cells, and increased production levels of Th_2 cytokines (4). Among parasitic diseases, it has been reported that eosinophilia is most frequently caused by helminth infections such as cystic echinococcosis (CE), fascioliasis, toxocariasis, schistosomiasis, strongyloidiasis, filariasis, and trichinellosis (3).

No study has examined the seropositivity of helminthic infectious agents in humans in the Black Sea region in the literature. This study aimed to determine the seropositivity rate of certain helminthic infections, such as CE, fascioliasis, toxocariasis, and trichinellosis, that were seen in Türkiye among eosinophilic and non-eosinophilic patients and investigate whether eosinophilia can be helpful as a biomarker for the diagnosis of helminth infections.

Materials and methods

Study Group

The population of the research was taken as the population of Samsun province. The population of Samsun province in 2019 was 1,348,542, and the male and female populations were approximately equal (Male population: 669,055 people and female population: 679,487 people) (5). The minimum sample size required for the eosinophilic group was calculated as 246 people within a 95% confidence level using the EpiInfo computer program (Centers for Disease Control and Prevention, Atlanta, USA), assuming an anticipated seropositivity of 20.0% and absolute precision of 5.0%. The non-eosinophilic group was calculated as 73 people, assuming an expected seropositivity of 5.0%

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and an absolute accuracy of 5.0%. To ensure gender representation, similar numbers of men and women within both groups were included in the study.

Based on this calculation a total of 646 sera were taken from patients (278 males and 279 females) with eosinophilia (>5%), and 89 samples were taken from patients (45 males and 44 females) without eosinophilia (<5%) which were referred to the Public Health Laboratory for hemogram analysis from Samsun province were included in the study. According to the hemogram test results, the patients were divided into two groups; those with a percentage of eosinophils >5% were defined as the "eosinophilic group" and those with <5% were defined as the "non-eosinophilic group". These two groups separated men and women, and patients were randomly selected to be included in the study. The sera of the patients were collected and stored at -20 °C until used.

Serological Tests

An in-house ELISA was used to investigate *F. hepatica*, *T. spiralis,* and *E. granulosus* antibodies in sera collected from the patients in the eosinophilic and non-eosinophilic groups (6-9). While interpreting the results of the ELISA test, the "serological index (SI)" formula was used, and the optical density (OD) value of each patient was used. Each plate was evaluated individually for anti-*Echinococcus* ELISA IgG and anti-*Trichinella* ELISA IgG. Negative, positive, and intermediate control sera were used on each plate. The mean and standard deviation of negative results were calculated. Results up to +2SD were considered negative, those between +2SD and +3SD were considered suspicious, and those greater than +3SD were considered positive. Antigen Unit (AU) evaluation intervals for Anti-*Fasciola* ELISA IgG: Negative < 10 < Intermediate value < 15 < Positive. Accordingly, patients with an intermediate AU were considered suspiciously positive, and those with an AU above 15 were considered positive.

An in-house Western blot was applied for *T. spiralis* and *T. canis* detection in sera (10, 11). Patients with specific "low molecular weight bands" (36-42 kDa) in *T. canis* WB analyses were considered positive. Of the positive and/or suspected positive sera by ELISA for *T. spiralis*, only those confirmed by WB were considered positive. For trichinellosis, only patients with three bands in the 52-70 kDa range, specific for infection, were evaluated as positive.

Statistical Analysis

The study data were analyzed with IBM SPSS v23. Conformity to normal distribution was examined with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Histograms, Q-Q plot tests, and the Chi-square and Fisher's exact tests were used to compare categorical variables according to groups. An independent samples t-test was used to compare normally distributed data, the Mann-Whitney *U* test was used to compare non-normally distributed data, and one-way analysis of variance (ANOVA) was used to compare normally distributed data between groups of three or more. Analysis results were presented as mean ± standard deviation, median, minimum–maximum value for quantitative data, and frequency (percentage) and 95% confidence intervals (CIs) for categorical data. The *P value* was considered significant if it was lower than 0.05.

Results

In the eosinophilic group (n = 557), seropositivity rates were 0.9% for echinococcosis, 0.5% for fascioliasis, 72.2% for toxocariosis, and 0.5% for trichinellosis, while they were 0.0%, 0.0%, 64%, and 1.1%, respectively, in the non-eosinophilic group (89 cases) (Table 1).

While no difference between males and females could be determined regarding E. granulosus, F. hepatica, and T. spiralis seropositivity, *Toxocara* positivity was statistically significantly more frequent in males ($P = 0.003$) (Table 2). There was no statistically significant relationship between seropositivity and age ($P > 0.05$) (Table 3).

One of the patients found positive for *T. spiralis* and *T. canis* together was in the non-eosinophilic control group. The *E. granulosus* ELISA titer was positive at 1/2.500 in two patients with *T. canis*, while it was positive at 1/640 in the third. Strong positivity in anti-*Fasciola* ELISA IgG strongly supports the preliminary diagnosis of fascioliasis. In addition, both *T. canis* and *T. spiralis* were positive in three patients using the Western Blot method. Since this method detects parasite-specific protein bands, cross-reactivity was not considered in these patients.

There were 406 (72.9%; 95%CI: 69.1-73.2%) people with at least one seropositivity in the eosinophilic group, while there were 57 (64.0%; 95%CI: 53.7-76.4%) people in the non-eosinophilic group. (p:0.085). In the eosinophilic group, the lowest mean eosinophil percentage was found in the *F. hepatica* suspected group with 7.6%, and the highest rate was found in the *E. granulosus* group with 9.9%. In the non-eosinophilic group, the lowest mean eosinophilia percentage was found in *T. spiralis* with 2.1%, and the highest rate in the *T. canis* group with 4.9% (Table 4).

Discussion

In many countries, including Türkiye, CE remains a significant public health problem and can cause life-threatening complications; it also imposes a significant burden on the economy due to loss of workforce and health expenditures (12,13). In our country, this rate is between 2.7% and 54.1% (14). In a study conducted in a tertiary hospital in Corum, which is in the Black Sea region, CE seropositivity was determined to be 5.7% (12). The low rate of CE positivity (0.8%) in our study may be because patients who applied to primary healthcare institutions and did not have CE-related complaints were included in

Table 1. Comparison of seropositivity rates between the eosinophilic and non-eosinophilic groups.

Test	Result	Eosinophilic group, (N = 557)		Non-eosinophilic group, (N = 89)		*P* value
		n	% (95% CI)	n	% (95 %CI)	
F. hepatica (ELISA)	Suspicious positive	10	1.8 (1.0-3.3)	1	1.1 (0.2-6.1)	N/A**
	Positive	3	0.5 (0.2-1.6)	0	0.0 (0.0-4.2)	
E. granulosus (ELISA)	Suspicious positive	52	9.3 (7.2-12.0)	5	5.6 (2.4-12.5)	N/A**
	Positive	5	0.9 (0.4-2.1)	0	0.0 (0.0-4.2)	
T. spiralis (WB)	Positive	3	0.5 (0.2-1.6)	1	1.1 (0.2-6.1)	0.896[F]
T. canis (WB)*	Positive	402	72.4 (68.6-76.0)	57	64.0 (53.7-73.2)	0.104

* *T. canis* WB of two patients in the eosinophilic group could not be studied because their serum samples were insufficient.
** This table does not meet Cochran's criteria.
F: Fisher's Exact test

Table 2. Comparison of seropositivity rates by gender.

Test	Result	Male (N = 323)		Female (N = 323)		*P* value
		n	% (95% CI)	n	% (95% CI)	
F. hepatica (ELISA)	Suspicious positive	9	2.8 (1.5-5.2)	2	0.6 (0.2-2.2)	N/A**
	Positive	2	0.6 (0.2-2.2)	1	0.3 (0.1-1.7)	
E. granulosus (ELISA)	Suspicious positive	34	10.5 (7.6-14.3)	23	7.1 (4.8-10.5)	N/A**
	Positive	2	0.6 (0.2-2.2)	3	0.9 (0.3-2.7)	
T. spiralis (WB)	Positive	2	0.6 (0.2-2.2)	2	0.6 (0.2-2.2)	>0.999[F]
T. canis (WB)*	Positive	248	76.8 (71.9-81.0)	211	65.3 (60.0-70.3)	0.002

F: Fisher's Exact test
* *T. canis* WB of two patients in the female group could not be studied because their serum samples were insufficient.
** This table does not meet Cochran's criteria.

Table 3. Comparison of mean ages by serological test results.

Test	Result	Mean ± SD	Median (min-max)	*P* value
F. hepatica (ELISA)	Suspicious positive (*n* = 11)	55.4 ± 15.8	54.0 (35.0 - 79.0)	0,673
	Positive (*n* = 3)	49.3 ± 21.1	61.0 (25.0 - 62.0)	
E. granulosus (ELISA)	Suspicious positive (*n* = 57)	49.9 ± 19.8	49.0 (18.0 - 87.0)	0,156
	Positive (*n* = 5)	36.6 ± 17.9	31.0 (21.0 - 66.0)	
T. spiralis (WB)	Positive (*n* = 4)	58.8 ± 21.4	67.5 (27.0 - 73.0)	0,278[U]
T. canis (WB)*	Positive (*n* = 459)	52.8 ± 16.3	54.0 (19.0 - 94.0)	0,076

U: Mann-Whitney U test.
* *T.canis* WB could not be studied because the serum samples of two patients were insufficient.

Table 4. Median eosinophil percentages of patients with suspicious positive or positive test results.

Test	Result	Eosinophilic group		Non-eosinophilic group	
		n	Median (min-max)	n	Median (min-max)
F. hepatica	Suspicious positive	10	7.6 (5.4 - 10.5)	1	2.5
	Positive	3	8.1 (7.4 - 8.6)	-	-
E. granulosus	Suspicious positive	52	8.9 (7.9 - 12.6)	5	3.1 (2.4 - 3.6)
	Positive	5	9.9 (7.8 - 12.1)	-	-
T. spiralis	Positive	3	8.7 (5.2 - 11.8)	1	2.1
T. canis	Positive	402	8.1 (6.5 - 9.0)	57	2.7 (1.0 - 4.9)

this research.

It has been reported that CE is generally seen in middle-aged people (40-59 years) and occurs slightly more frequently in males than in females, which could be explained by males' higher exposure risks due to occupation. Still, there are also controversial results (12, 15). In our study, gender was not significant, and it was observed that the mean age of the patients (36.6) corresponded to a slightly younger period.

Eosinophilia can be seen in 19-80% of cases in CE, depending on the localization of the cysts, leakage status, or concomitant parasitic infection (12). Although

eosinophilia has limited value in diagnosing and screening CE, it can be used as an essential tool in the follow-up and evaluation of the patient's prognosis after treatment. Cappello et al detected hypereosinophilia in 56.3% of patients, and this significant ratio decreased to 12% after albendazole treatment (16). In our study, although CE seropositivity was found to be higher in the eosinophilic group (0.9%) than in the non-eosinophilic group (0.0%), no statistical difference was found ($P = 0.336$).

Serological tests such as indirect hemagglutination (IHA), ELISA, and WB can support radiological findings, especially in the differential diagnosis of unilocular and multilocular cysts, and in the follow-up of recurrences after surgery (7,15). However, the sensitivity and specificity of the tests may vary due to factors such as the type of antigen used, the localization of the cyst, its viability, and the parasite strain (17). ELISA, which we also used in our study, is a test that is easy to prepare, relatively low-cost, and has a sensitivity and specificity of about 95% (18).

Serological studies are the primary diagnostic tool in diagnosing fascioliasis and aid in diagnosing asymptomatic cases or the acute phase of the disease, even before parasite eggs can be observed in the stool (19). The specificity of ELISA is 95-100%, and the sensitivity is 93-97%, especially when using the excretory/secretory antigens of adult *F. hepatica* (1,2,8,9,20,21). Although data on the spread of fascioliasis in humans in Samsun and its surroundings are limited, the seropositivity found in almost 1/3 (32.4%) of sheep (22) may also guide the situation in humans. Again, the report of a case in Giresun in which fascioliasis was detected incidentally by endoscopic retrograde cholangiopancreatography (ERCP) (23) and the serological positivity of three (0.5%) cases in our study suggests that the disease poses a problem for the people of the Black Sea Region, and a substantial number of asymptomatic cases are missed. Kaymak Cihan et al detected *F. hepatica* IHA positivity at a titer of 1/5120 in a boy who lived in a rural area who had no other complaints except occasional abdominal pain but was hospitalized for severe eosinophilia, and the eosinophil count decreased significantly after treatment (24). Although parasitic eggs can be seen in the chronic phase of *F. hepatica* infection, they may sometimes be undetected due to low numbers, intermittent excretion, or ectopic localization. In this respect, eosinophilia, which persists throughout the chronic phase in 14-82% of patients, may be an essential marker for the disease (24).

F. hepatica seropositivity was determined as 73.7% and 35%, respectively, in eosinophilic and non-eosinophilic cattle in Eastern Anatolia using the ELISA (25). In Isparta and its surroundings, Demirci et al (1,2) found the difference in positivity rates between eosinophilic and non-eosinophilic patient groups to be statistically similar in 2002 but different in 2003. Although three seropositive cases were detected only in the eosinophilic group, no statistical difference was found in our study.

It has been reported that fascioliasis is more common in women and people around the age of 40 (20). The mean age of the three seropositive cases found in our study was around 49 years, and although two of them were men, no statistical significance was found according to gender or age. Prolonged and repeated exposures to parasites cause eosinophil stimulation by T lymphocytes, producing cytokines such as IL-4, IL-5, and IL-13 (4). In the presence of severe eosinophilia, fascioliasis should be kept in mind, especially if there is a history of sheep rearing and spring water use in rural areas (20,24).

In our country, the prevalence of *toxocara* varies between 7.6 and 51.35% and is higher in children and those living in rural areas (11,26-28). The seropositivity detected in our study was extreme (71.1%) compared to other studies. *The* excessive rate (64.0%), even in the non-eosinophilic group, indicates a considerable presence of toxocariasis in the region. The special climate of the Black Sea region with high relative humidity and soil moisture might create a suitable environment for the survival of *Toxocara* eggs.

Although there are studies to the contrary, people living in rural areas and suburbs are at higher risk than those in urban areas due to increased exposure to soil, livestock, stray cats and dogs, lack of education or knowledge, failure to follow hygiene rules, and inadequate infrastructure (11,26-28). Samsun is a province of Türkiye which has a relatively high number of villages, and Vezirkopru, located in the Samsun countryside, is the district with the highest number of villages in Türkiye. These settlement preferences could be a reason for the high seropositivity rate of toxocariasis.

Similar to our findings, Toxocara seropositivity is more common in males worldwide, including in our country (11,26,28). It is emphasized that toxocariasis is more common in children due to playing with contaminated soil in playgrounds, eating soil, and poor hand-washing habits. On the other hand, consumption of contaminated vegetables or undercooked meat is thought to be a factor that increases the parasite burden over the years (26,28). Although the mean age of the seropositive patient group in our study was higher, no relationship was found between seropositivity and age in our study.

It has been reported that *T. canis* seropositivity reaches 87% among asymptomatic eosinophilic patients of unknown cause and should be considered in the differential diagnosis of idiopathic hypereosinophilic syndrome (27). In Türkiye, both toxocariasis and fascioliasis seropositivity were significantly higher in the eosinophilic patient group (1,2,28). In our study, *Toxocara* seropositivity was relatively high in both eosinophilic (72.4%) and non-eosinophilic groups (64.0%) without statistical significance. It could be explained by the cross-reactivity of the ELISA with other helminthic diseases (1,24,27). Still, it was excluded due to using both ELISA

and WB, which are specific for toxocariasis.

Since the end of the last century, the number of human trichinellosis cases has increased significantly in the Middle East and Balkan countries. The high amount of sausage and salami processed from pork and consumed raw in Europe, such as eating meat undercooked, increases the incidence of trichinellosis. In addition to pigs, it can also be transmitted by horses, cattle, bears, and even sheep (29). Trichinellosis was found at a rate of 2.1% in beef and 1.4% in mutton meat sold in markets in China, and the mixing of rats, which consume the internal organs of wild boars hunted in the wild, into the feed of farm animals could be a factor in this contamination (30). Although eosinophilia occurring early in the infection is an essential parameter in the surveillance and screening of trichinellosis infections, it is not sufficient alone (29,31). *Trichinella* seropositivity was investigated by ELISA and WB in 1,342 eosinophilic individuals by Sviben et al; positivity was detected with both tests in 3.65% of the participants (31). Although no difference was found according to gender, the increase in infection with age was significant.

Pork consumption is extremely low in our country, where most of the population is Muslim (32). On the other hand, several trichinellosis outbreaks have been described in Izmir and Bursa provinces due to consuming raw foods with pork products put on the market by unregistered and uncontrolled enterprises (33,34). In the epidemic related to the consumption of raw pork meatballs in Izmir, the infection was confirmed serologically in 47 (62%) of 76 children suspected of trichinellosis. In the epidemic in Izmir, it was stated that eosinophilia was the most prominent laboratory finding in 88% of the cases. Positivity of anti-*Trichinella* IgG antibodies was reached in 73% of the cases. The source of the outbreaks may originate from wild boar, pig farming, or horse meat consumption (33-36). In our study, for the first time from Samsun and its region, four cases (0.6%) were found positive with both ELISA and WB, but age, gender, and having eosinophilia were non-significant ($P > 0.050$). In our country, no study reflects the seroprevalence status except for studies during epidemics. Compared to other countries, the low seropositivity rate (0.5%) found in our research can be explained by Islamic beliefs and culture. On the other hand, detecting four seropositive cases raises questions about the possibility of uncontrolled meat production in our region. Considering that some cases can be missed despite efforts for confirmation with serological tests (37-40), there may be more people with trichinellosis in our region.

Our study's limitations include the lack of information, such as the patients' clinical complaints, the drugs they use, imaging results, parasite results in the stool, their occupations, and their environment. Another handicap is keeping the number of non-eosinophilic groups low due to budget constraints.

Conclusion

As a result, with this study carried out for the first time in Samsun and its region, seropositivity of fascioliasis in three cases and trichinellosis in four cases was found in local people. The seropositivity of toxocariasis (exceeding 70%) is relatively high compared to other regions in our country. It can be stated that hydatid cysts still exist in our region at a rate close to 1%. No difference was found between eosinophilic and non-eosinophilic groups regarding echinococcosis, fascioliasis, toxocariasis, and trichinellosis positivity. Although the results suggest that helminth infections may be relatively rare causes of eosinophilia in our region, more data are required due to the low number of controls. For this purpose, a comprehensive seroprevalence study with a larger control group is planned, and other helminthic agents such as ascariasis, strongyloidiasis, and hookworm infections are investigated.

Authors' Contribution

Conceptualization: Aysegul Taylan Ozkan.
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Writing–original draft: Aysegul Taylan Ozkan.
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Competing Interests

The authors declare no conflict of interest.

Ethical Approval

Not applicable.

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