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Seropositivity of anti-toxocara canis IgG in fibromyalgia patients

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ÖZET

Fibromiyalji Hastalarında Anti-Toxocara canis IgG Seropozitifliği

Toksokariyazis, köpeklerin yuvarlark kurtları olarak bilinen Toxocara canis'in (T. canis) larva formlarının denen olduğu helmintik bir zoonozdur. Fibromiyalji (FM) prevalansı, HIV, hepatit B ve C virüsü (HBV ve HCV) enfeksiyonları ile Lyme hastalığı olan bireylerde daha yüksektir. Ancak literatürde daha önce T. canis ile FM arasında olası bir ilişki belirtilmemiştir. Bu çalışma FM hastalarında T. canis seropozitifliğini belirlemeyi amaçlamaktadır.

Çalışmaya, Fizik Tedavi ve Rehabilitasyon kliniğinden 91 FM hastası dahil edilmiştir. Toplanan kan örneklerinde eozinofil sayısını belirlenmiş ve Toxocara-IgG ELISA kiti kullanılarak T. canis lavrasından salınan/salgılanan antijenlere karşı üretilen antikorlar araştırılmıştır.

91 FM hastasından 11'inde (%12,08) T. canis antijeni pozitif bulunmuştur. Çalışmamızda, seropozitiflik ile eğitim düzeyi, günlük el yıkama alışkanlığı arasında negatif bir korelasyon bulunmuştur. Evcil hayvan olarak köpeğe sahip olma durumu ile sahip olma süresi de prevalansı arttırmıştır. Bulgularımız toksokariyazis ile FM etiyolojisi ile arasında olası bir ilişkiyi göstermekle birlikte, daha geniş hasta gruplarında vaka kontrollü çalışmalara da ihtiyaç duyulmaktadır.

Anahtar Kelimeler: *Fibromiyalji, Toxocara canis, Toksokariyazis, seropozitiflik*

SUMMARY

Toxocariasis is a helminthic zoonosis caused by larval stages of Toxocara canis (T. canis), the roundworms of dogs. The prevalence of fibromyalgia (FM) is found higher in patients with Lyme disease, HIV, hepatitis B (HBV) and C viruses (HCV) infections. However, a possible relationship between T. canis infection and FM has not been reported previously in the literature. We carried out a study in order to evaluate the T. canis seropositivity in FM patients.

91 patients with FM were recruited from the Physical and Rehabilitation Medicine outpatient clinic. Blood samples were obtained to determine eosinophil and total IgE levels with the ELISA method using the Toxocara-IgG ELISA kit to detect antibodies against the excretory/secretory antigen of the T. canis larvae.

11 of the FM patients out of 91 (12,08%) had positive test results for T. canis seropositivity. We have found negative correlation between seropositivity and education level, daily hand washing habit. Having a dog as a pet and the duration of owning also increased prevalence. Our findings may be a sign of a possible role of toxocariasis in the etiology of FM, but needs to be investigated by case-control studies with larger populations.

Key words: *Fibromyalgia, Toxocara canis, toxocariasis, seropositivity*

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Introduction

Toxocariasis is a helminthic zoonosis caused by larval stages of *Toxocara canis* (*T. canis*) and, less frequently, by *Toxocara cati* (*T. cati*), the roundworms of dogs and cats, respectively. Humans may accidentally ingest embryonated eggs containing the infective larvae which are released in the upper small intestine and then pass through the intestinal epithelium to reach the blood vessels, where they can migrate to the different visceral organs and tissues of the body (1). The spectrum of the clinical manifestations in toxocariasis varies widely from asymptomatic cases to systemic infections (2). According to the affected organ, there are two clinical appearances of toxocariasis. These are "Visceral Larva Migrans (VLM)" involving various organs and "Ocular Larva Migrans (OLM) which is limited to the eye. Additionally, there is a recent term "Occult Toxocariasis (OT)" that defines a clinical syndrome having non-specific clinical and laboratory findings (1).

Although definite diagnosis is made by seeing the larvae in the affected tissues on histological evaluation, usually this is not possible. Diagnosis of toxocariasis is often made by detecting the IgG antibodies specific to T. canis in patient's serum with enzyme linked immunosorbent assay (ELISA) (3).

On the other hand, fibromyalgia (FM) is a syndrome with an unknown etiology, characterized by chronic, widespread musculoskeletal pain with tenderness over specific trigger points, fatigue, morning stiffness, and sleep disturbance (4). Various etiological factors have been suggested to trigger FM, including systemic rheumatismal diseases, physical trauma, psychological disorders, and chronic infections (5, 6). The prevalence of FM is found higher in patients with Lyme disease, HIV, hepatitis B (HBV) and C virus (HCV) infections (7). However, to our knowledge, a possible relationship between T. canis infection and FM has not been reported previously in the literature. To add further information and to increase the awareness of the physicians about toxocariasis, we carried out a study in order to evaluate the *T. canis* seropositivity in FM patients.

Material and Method

All patients were informed about the study procedure and gave written consent to participate. The Local Ethics Committee approved the study protocol and it was designed as a descriptive study. 91 patients with FM (who met the 1990, American College of Rheumatology criteria for the diagnosis of FM) (8) were recruited from the Physical and Rehabilitation Medicine outpatient clinic in one-year's period. Demographic features of the participants (age, educational status, number of daily hand washing, having dog as a pet and duration) were

noted. Blood samples were obtained to determine eosinophil and total IgE levels. Sera obtained from the blood samples were kept at -20 °C until the anti Toxocara IgG study. Stool samples were also obtained from all subjects and examined for other nematodes to eliminate possible cross-reactions. The stored sera were studied with the ELISA method using the Toxocara-IgG ELISA kit NovaLisa NOVATEC (Immundiagnostica GMBH, Germany) to detect antibodies against the excretory/secretory antigen of the T. canis larvae. The results were evaluated with the kit procedure that was suggested by the producing company.

Considering the probability of cross-reaction with other nematodes, parasite egg was searched to exclude the existence of other parasite eggs for the consecutive three days in the stool samples of the participants who had been detected as positive for T. canis IgG with ELISA.

Statistical analysis was done using SPSS (Statistical Package for Social Sciences) for Windows, version 16.0. Statistically significance level was set to $p<0,05$.

Results

All the participants were female. Eleven of the 91 patients (12,08%) had positive test results for *Toxocara*. *Toxocara canis* positivity distribution according to the demographic features of the FM patients is shown in Table 1. We have found higher seropositivity in individuals with being less educated, washing the hands less, having a dog as a pet and the longer duration of having a dog as a pet. We have found higher seropositivity in individuals with the complaint of musculoskeletal pain, with hypereosinophilia and leukocytosis. *Toxocara canis* IgG positivity distribution according to the patient complaints and laboratory findings is shown in Table 2. None of the participants had increased liver functions or IgE levels.

Table 1. *Toxocara canis* positivity distribution according to the demographic features of the FM patients.

DEMOGRAPHIC FEATURE	*Toxocara canis*		Total	Statistics
	Seropositive (%)	Seronegative (%)		*P value*
Age				0.212
15-34	8.3	91.7	36	
35-54	9.1	90.9	33	
55 and more	22.7	77.3	22	
Educational Status				0.003
Primary School	33.3	66.7	24	
Middle School	9.1	90.9	11	
High School	3	97	33	
University	4.3	95.7	23	
Number of Daily Hand washing				0.0001
1-5	81.8	18.2	11	
6-9	1.6	98.4	64	
10 and more	6.2	93.8	16	
Having Dog as a Pet				0.0001
Yes	66.7	33.3	6	
No	8.2	91.8	85	
Duration of Having Dog as a Pet (Months)				*
1-12	8	92	87	
13 and more	100	0	4	

* Due to Cochrane principles, x^2 analysis could not be applied because of the 0 (zero) value.

Table 2. *Toxocara canis* IgG positivity distribution according to patient complaint and laboratory findings.

PATIENT COMPLAINT	*Toxocara canis*		Total	Statistics
	Seropositive (%)	Seronegative (%)		*P value*
Weakness/Fatigue				0.0001
Yes	34.5	65.5	29	
No	1.6	98.4	62	
LABORATORY FINDINGS				
Increased eosinophilia				*
Yes	100	0	6	
No	5.9	94.1	85	
Existence of Leukocytosis				0.0001
Yes	50	50	6	
No	6.3	93.7	85	

* Due to Cochrane principles, x^2 analysis could not be applied because of the 0 (zero) value.

Discussion

T. canis is a common parasite of dogs living close to humans. Infection occurs with consumption of food and drinks contaminated with embrionated eggs. The risk of getting these eggs from the soil is higher in children with geophagia. Public parks, children playgrounds and streets are highly contaminated with toxocara eggs, as people walk their pets routinely in these places (9-12).

Living in rural areas and having low income are associated with high toxocara seropositivity rates. Dog shelters and pet-shops are potential source for mature toxocara. Similarly, having a dog as a pet inside the house has been defined as a risk factor (10).

In this study, we have tried to explore the *T. canis* IgG positivity in FM patients which was found 12,08% of the FM patients. The incidence and prevalence of T. canis infection in humans is not exactly known yet (13), but there are several studies reporting that the seroprevalence of toxocariasis varies between 1.8 - 58.3% depending on country, study group and socio-cultural level (14-16). Previous studies report toxocariasis prevalence in healthy subjects between 2.6-6% in Turkey (17, 18). Given this, we have found twice as much higher seroprevalence in FM patients in Turkey. This may be a sign of a possible role of toxocariasis in the etiology of FM, but needs to be investigated by case-control studies with larger populations.

Ocular toxocariasis (OT) is a clinical manifestation of toxocariasis that is less well defined and frequently undiagnosed but it can commonly occur. OT is characterized by nonspecific signs and symptoms that do not fall into the category of classic VLM or OLM. OT is an organ oriented immunopathological host response to continued stimulation of the host immune system by parasite antigens (19). The clinical expression varies from a pulmonary involvement such as asthma and acute bronchitis (20), to dermatological disorders such as chronic urticaria (21), lymphadenopathy, myositis and to a pseudorheumatic syndrome such as arthralgia and myalgia (22).

In an animal study, Chieffi et al (23) showed that *T. canis* causes a decrease in muscular strength. We also found that seropositivity was higher in individuals having the complaint of weakness and fatigue. Additionally, we have found that all *T. canis* IgG positive patients had musculoskeletal pain for more than 12 months.

In our study, we have found that the highest seropositivity was above the age of 55, which is in line with the previous literature (10, 24). We think that this is due to the decrease in hygiene with the increasing age and increased risk of exposure to the agent with the increased lifetime.

The present study also supports the studies that have reported that seropositivity increases with low level of education (25-27) and low income (28-30). Reasons for this may be the following: lack of adequate infra-structure and the high number of un-controlled dogs in the environment of individuals with low income, ignoring hygiene due to low level of education.

We have seen that seropositivity decreases with the increase in the number of daily hand washing, which have been also previously shown by several studies (23, 31, 32). Inadequate hygiene increases the risk of toxocara infection.

The ELISA test using TES antigens is the most common diagnostic method to detect anti-toxocara IgG antibodies (33), but it still remains problematic considering polyparasitism as the possibility of cross-reactions is high, which reduces its diagnostic value. False positive results may occur in patients with other parasites such as ascariasis, strongyloidosis and trichinellosis (9). To overcome this problem we searched parasite egg to exclude the existence of other parasite eggs for the consecutive three days in the stool samples of the participants that had been detected as positive for *T. canis* IgG with ELISA. No other parasite eggs were encountered in any of the individuals' stool samples.

The relationship between helminth infections and hypereosinophilia is well-known. Hypereosinophilia is commonly detected in blood and tissues of patients with toxocariasis. But one should not ignore the diagnosis of Toxocara even if the level of eosinophilia is within normal limits, because in OT some patients do not present hypereosinophilia (9, 34, 35). On the other hand, IgE increases in parasite diseases, atopic diseases and some immunodeficiency conditions. Similar with the other studies we have found higher seropositivity in individuals with hypereosinophilia and leukocytosis (14, 19, 28, 36).

Good hygiene and public education plays a major role in preventing toxocara infections. To reduce human exposure, puppies should be dewormed. Feces should be removed from areas where children play. Hands should be washed before eating. Additionally, children should be taught not to eat soil, and to wash their hands after playing with pets and after outdoor activities. In severe cases, anthelmintic drugs should be used.

In the light of the results of our study, we can state that there may be a possible relationship between toxocariasis and FM, which needs to be uncovered.

Conclusion

The present study reports that low education status, less-frequent hand-washing, having a dog as a pet, positively related with time can be risk factors for *T. canis* IgG positivity in patients with FM. This may be a sign of a possible role of toxocariasis in the etiology of FM, but needs to be investigated by case-control studies with larger populations. And, further investigation with larger number of samples is warranted to evaluate the high seroprevalance in FM patients and enlighten the possible role of *T. canis* as a cofactor in the etiology of FM.

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