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Toxocara Canis IgG Seropositivity in Patients with Chronic Urticaria

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ABSTRACT

We aimed to investigate IgG antibody levels specific to *Toxocara canis* (*T. canis*), a parasite which subsists in dog's intestine, on serum samples obtained from patients with chronic urticaria (CU) to evaluate effective risk in CU etiopathogenesis.

In this study, 73 patients diagnosed with CU and 109 healthy individuals as control group, were included. Various factors such as sex, age, education and income, daily hand washing habits, history of dog owning and soil eating were questioned in patient anamnesis. *T. canis* IgG antibodies were detected using an enzyme linked immunosorbent assay (ELISA) kit prepared with *T. canis* larval excretory-secretory antigens. Positive results were confirmed with western blot (WB) WB test.

We found *T. canis* IgG positivity in 17.8% (n=13) of patients (n=73) with CU. But we did not observe any *T. canis* IgG positivity in healthy controls (n=109). Low molecular weight bands (24-35 kDa) were observed in 11 samples in WB analyses while two of the samples were weakly positive. It is revealed that dog owning history increases *T. canis* seropositivity 12.9 times while insufficient daily hand washing habit (less than six times a day) increases seropositivity 20.7 times. Our study showed that *T. canis* may trigger CU since we found 17.8% seropositivity in 73 patients with CU and none in 109 healthy individuals.

Moreover, various socio-demographic characteristics have been shown to affect *T. canis* seropositivity in patients with CU.

Keywords: Chronic; IgG; Seropositivity; Toxocara canis; Urticaria

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. Accidentally, humans ingest embryonated eggs containing the infective larva 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 different visceral organs and tissues of the body.[1]

The spectrum of the clinical manifestations in toxocariasis varies widely from asymptomatic cases to

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systemic infections.[2] According to the affected organ, there are two clinical appearances of toxocariasis. These are "Visceral Larva Migrans (VLM)" involving organs and "Ocular Larva Migrans (OLM) that 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.[1-4]

Urticaria is a pathological condition characterized with erythematous, edematous blisters that are itchy with temporary rashes. It is a common disease that is seen in 15-25% of humans in at least one part of their lifetime. Urticaria is classified as acute or chronic according to its duration. Clinical findings above six or more weeks are defined as CU. 75% of the urticaria cases are idiopathic. Drugs, food and helminthic infections seem to be the etiological factors. It is reported that urticarial lesions are observed in approximately 80% of parasitosis cases.[5,6]

We aimed to investigate Toxocara specific IgG antibody levels from serum samples obtained from patients with CU with enzyme linked immunosorbent assay (ELISA) method, and to confirm positive results with western blot (WB) test, in order to determine the Toxocara and to evaluate effective risk in CU etiopathogenesis.

PATIENTS AND METHODS

Patients

Seventy-three patients, diagnosed with CU in GATA Haydarpasa Hospital, Immunologic and Allergic Diseases Service were included in the study. In order to determine the risk factors for Anti-Toxocara canis IgG seropositivity, patients' age, sex, education, income, daily hand-washing habits, dog feeding and previous soil-eating histories were collected.

Following the ethical board recommendations, 5 ml blood samples were collected from the patients during diagnostic processess. After separation of blood serum, eosinophil and IgE levels were investigated. Remaining serum samples were frozen at-20°C until serological tests and thawed only once for the tests.

ELISA and WB Tests

Anti-*T.canis* IgG antibodies investigated according to the instructions of NovaLisa Novatec (Immundiagnostica GMBH, Germany) ELISA kit, prepared with *T. canis* larval excretory-secretory (TES) antigens of *T. canis* larvae. WB analyses (Toxocara WB IgG, Ldbio Diagnostics, France) with TES antigens were performed to confirm the samples which IgG antibodies were found positive on ELISA.

Statistical Analysis

Statistical analyses of the study data were evaluated using SPSS for Windows 16.0 program. Along with complementary analyses (frequency, standard derivation (SD), percentage, mean), Pearsons's chi-squared test were used. In order to evaluate factors effecting *T. canis* IgG positivity, logistic regression analyses were used. *P* values below 0.05 were accepted as statistically meaningful.

Evaluation of Parasite Eggs in Feces Samples

In order to eliminate cross reaction possibility because of patient contact with nematodes other than *T. canis*, which may also give IgG antibody positivity in the serum, feces samples were collected from patients for consecutive three days and evaluated for other nematode eggs.

RESULTS

Seventy-three patients, diagnosed with CU were included in the study. 57.5% (n=42) were male while 42.5% were female. 38.4% (=28) were 15-35 years old, 42.5% (n=31) were 36-65 years and 19.2% (n=14) were above 56 years. Other socio-demographic variants and related laboratory findings were listed on Table 1.

T. canis IgG antibodies were detected on 13 (17.8%) of 73 patients, diagnosed with CU. WB analyses were performed to this 13 patients to confirm ELISA results. At least two low molecular weight (LMW) (24-35 80 kDa) bands were detected on the gel electrophoresis which indicated *T. canis* with high specificity along with less specific high molecular weight (HMW) (>80 kDa) bands on 11 patient samples while specific LMW bands were very strong while less specific, HMW bands were weakly positive on two patient samples. Distribution of *T. canis* specificity according to the various demographic attributions and laboratory data were presented in Table 1. We found that washing hands five or less times in a day increases seropositivity 20.7 times in CU patients while dog feeding history increased seropositivity 12.9 times (Table 2).

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Table 1. Distribution of *T. canis* antibody positivity according to age, sex, education, monthly income, daily hand washing habit, soil-eating and dog feeding history, hypereosinophilia and IgE levels.

Demographic and laboratory findings	Negative *T. canis* IgG n(%)	Positive *T. canis* IgG n(%)	*P* value
Sex			0.119
Male	32 (76.2)	10 (23.8)	
Female	28 (90.3)	3 (9.7)	
Age			**0.002**
15-35	24 (85.7)	4 14.3)	
36-55	29 (93.5)	2 (6.5)	
56 or above	7 (50)	7 (50)	
Education level			*
Elementary school or below	10 (71.4)	4 (28.6)	
Secondary school	4 (100)	0 (0)	
High school	24 (82.8	5 (17.2)	
University or higher	22 (84.6)	4 (15.4)	
Monthly income			0.667
900 TL or below	15 (78.9)	4 (21.1)	
901 TL or above	45 (83.3)	9 (16.7)	
Hand washing / day			*
1-5/ Day	3 (23.1)	10 (76.9)	
6-9/ Day	42 (93.3)	3 (6.7)	
10 or above / Day	15 (100)	0 (0)	
Soil-eating history			*
Yes	0 (0)	4 (100)	
No	60 (87)	9 (13)	
Dog-feeding history			**0.001**
Yes	2 (33.3)	4 (66.7)	
No	58 (86.6)	9 (13.4)	
Hypereosinophilia			**0.0001**
Yes	3 (27.3)	8 (72.7)	
No	57 (91.9)	5 (8.1)	
High IgE level			0.699
Yes	3 (75)	1 (25)	
No	57 (82.6)	12 (17.4)	

*According to Cochran rules, x^2 analyses could not be done because the observed values are 0 (zero).

Table 2. Factors effecting *Toxocara canis* positivity

Demographic and laboratory findings	OR	%95 Confidence Interval	*P* value
Hand washing / day	20.727	2.522-170.364	0.005
Dog-feeding history	12.889	2.053-80.903	0.006

Table 3. Distribution of *T. canis* seropositivity in some studies outside of Turkey

Writer, Year, Location	Study Results
Zwolinski et al. (11), 2000, Poland	Seropositivity was found in 40.1% of Toxocara suspected patients. It was 44.2% positive in males, 36.5% in females; 21.2% in 16-30 year-old age group, 37.5% in 31-45 year-old age group and 47.8% in 45+ year-old age group; 56.1% in people living on rural areas, 30.9% in people living in small towns and 13% in people living in cities.
Chiodo et al. (14), 2006, Argentina	Seropositivity was 23% among volunteers; which was 26% in men and 20.3% in women; 23% in people who feed dogs at home while 0% in people who do not; and 86.9% in patients with eosinophlilia while 37.6% in healthy people.
Rubinsky-Elefant et al. (23), 2008, Brasil	Seropositivity was 26.8% in volunteers, which was 33.7% in uneducated people, while 28.2% in 1-4 years, 24.1% in 5-8 years and 17.6% in people who were educated for 8 or more years; 32.6% in people with low income while 11.3% in people with higher income; 28.5% on dog feeding people while 20.2% in people who do not feed dogs.
Stensvold et al (20), 2009, Denmark	Seropositivity was found 2.4% on Toxocara suspected patients; which was 5.1% in men while 2.1% in women.
Roldan et al. (18), 2009, Peru	Toxocara seropositivity was found 53.1% among randomly selected patients; which was 71.3% in menwhile 28.7% in women; 93.9% in people who fed dog or cat at home while 6.1% in people who did not, 80% in people with soil-eating history and 20% in persons without the historyin persons without the history.
Romano et al. (19), 2010, Malaysia	Seropositivity was found 4.8% in randomly selected patients; which was 9.5% in men while 1% in women; 6.3% in children below 12 years old while 1.2% above 13 years old.

Table 4. Distribution of *T. canis* IgG seropositivity on some studies within Turkey.

Writers, Year	Study Results
Büyükbaba et al. (10), 1996	Of 177 children, aging between 1-10 years and living in İstanbul, seropositivity was 47.3% among those living on rural areas and 11.9% among those living in cities.
Yazar et al. (15), 2010	Among 112 hospitalized patients, seropositivity was 21.4% which was 27.8% in men while 13.7 in women; highest seropositivity (30%) was seen in younger people aging 11-20 years old while lowest (12%) was in people aging above 44.
Doğan et al. (25), 2007	In this study which was conducted on 571 children, living in Northwest of Turkey, seropositivity was found 12.95%; which was 16.9% in people living in rural areas, while 0.71% in people living in cities and 12.3% in people who fed dogs at home while 4.6% in people who did not.
Demirci et al. (22), 2002	Seropositivity was 29.1% in patients with eosinophilia (n=134) while 19.4% in patients without eosinophilia (n=134) and 15.5% in healthy control group (n=84)
Karadam et al. (27), 2007	In this study, conducted on 700 patients, seropositivity was 32.6% in patients with eosinophilia (n=350) while it was 20.3% in patients without eosinophilia (n=350).

DISCUSSION

Toxocara eggs are released into the environment by cat or dog feces. Larvae develop within the eggs in soil which can be taken orally by humans with contaminated food or drinks. Larvae leave the eggs in intestine and passes to the blood vessels through intestinal epithelia. Larvae cannot reach mature phase within humans because of unfinished lung development. Larvae cause damage on tissues they enter.[1-4, 7-9] Even though diagnosis can be truly confirmed with visual detection of larvae on histopathological evaluation, however it is not generally possible. Diagnoses are usually confirmed with detection of IgG antibodies in patient serum with ELISA, and WB.[3,10]

Skin symptoms are usually observed as chronic itching, CU and eczema types. Less common symptoms are hypodermia, vasculitis, eosinophilic folliculitis, Reiter and Wells syndromes.[11] New researches indicate that skin symptoms may be the only clinical symptoms of the disease.[12] According to some literature, hypereosinophlia is the main reason of the skin symptoms which is explained by the release of cutaneous chemotactic factor with the effect of eosinophils. Another idea is the histamine release caused by the proteinase activity of TES antigens.[13]

Incidence and prevalence ratios of *T. canis* infections are still not known in humans. However, sero-epidemiological studies show varying results depending on the population and study area.[14,15]

Studies investigating toxocariasis on CU patients are very limited.[6,12,16] A possible relation between antibodies against *T. canis* and CU and eczema has been proposed in previous studies but could not clearly demonstrated. Demirci et al.[6] found that *T. canis* antibody positivity was 29% (n=62) on CU patients in their study in 2003. Humbert et al.[12] was investigated the relation of *T. canis* antibody seropositivity with various skin problems in 653 patients during 1994-95 years. T canis antibodies were found positive in 38.1% of21 prurigo patients, 15.4% in 52 patients with itching problems, 19.5% in 128 CU patients and 18.6% in 72 eczema patients which indicated significant relation between Toxocara antibody positivity and prurigo and urticaria. Wolfrom et al.[16] found Toxocara antibody seropositive in 65% of 33 patients with CU in 1996. Our findings, with the Toxocara seropositivity found 17.8% on 73 CU patients, demonstrated similar results with the previous studies.

Numerous studies investigated *T. canis* seropositivity and related factors in various patient groups and healthy individuals which were summarized in Table 3 and Table 4. Seropositivity rate was found much higher in men (23.8%) than women,[7,9] in our study like many other studies.[15,17-21] *T. canis* seropositivity was found much higher in elderly, which was similar to other studies.[11,12] The reason behind this phenomenon could be longer exposure chance of elders to the antigen and/or decreasing sanitation habit of old people.

Increasing education level or time helped decrease seropositivity, which was also similar to other studies.[21,23]

Living in poor regions, are more correlated with Toxocara seropositivity than having mediocre or higher income [1,7--9] Our findings like other studies[21,23,24] showed more seropositivity in patients with lower income compared to patients with higher income.This could be because of inadequate infrastructure on the living areas, ignoring personal sanitation possibly because of low education and living in rural areas or suburbs which contain more stray cats and dogs.

Higher hand washing numbers helpsto decrease *T. canis* seropositivity.[17,21] We found washing hands five or less times in a day increased *T. canis* seropositivity 20.7 folds. It could easily be connected to the elimination of infectious eggs from hands with frequent hand washing habit.

Parks and green areas inside the cities can be highly contaminated with Toxocara eggs because of walking pets. Dog shelters and pet shops could be the nesting place for adult forms of Toxocara. Feeding cat or dog at home or soil-eating habit are also important risk factors for toxocariasis.[1,7-9] Our findings which are correlated with other studies[21,24-26] demonstrated *T. canis* seropositivity were much higher in patients with dog feeding history than the others which were statistically 12.9 fold increasing risk factor.

Risk of ingesting soil containing embryos is higher in children with a soil-eating history.[1,7-9] Similar to others,[26] we found that all the patients with soil eating history were seropositive while it was only 13% in the other patients.

Relation between helminthic infections and eosinophilia is known for years. Toxocariasis patients develop eosinophilia in blood or tissues. However, having normal eosinophil counts do not exclude toxocariasis.[17,22,27] Total serum IgE level increases in some parasitic diseases, atopic diseases and immune deficiency conditions. Total IgE levels of toxocariasis patients also increases in most cases.[28] *T. canis* seropositivity were 72.7% in CU patients with eosinophilia and 25% in CU patients with high total IgE which were higher than patients without these symptoms (8.1% and 17.4%, respectively) which was also similar to other studies.[17,18,27,29]

Most frequently used diagnostic method for toxocariasis is to demonstrate antibody existence in serologic tests. However, other helminthic diseases such as ascariasis, strongiloidosis, filariasis, anisakiasis and fascioliasis may give cross reactions because of antigen similarity.[30] TES antigen recently becomes more preferred in serological diagnosis of toxocariasis

because of being more specific with its LMW components which also eliminates cross reactions. WB test with TES antigens (TES-WB) is the most specific method of detecting Toxocara antibodies. Best effective serological diagnosis method is to confirm seropositivity with TES-WB after scanning TES-IgG antibodies with ELISA.[18,30] ELISA method was used in most studies without confirming with WB.[14,18,19,25,27] In a study by Ozkoc et al.,[31] 12 (5.7%) of 209 patients became positive in ELISA for Toxocara IgG but only five of them were confirmed positive in Toxocara IgG-WB. Also three of the patients who were considered negative on initial ELISA tests showed HMW bands on WB.[31] Similar to this study, we were able to confirm only 11 of the 13 ELISA positive patients while only two samples showed LMW bands along with HMW bands on WB (Figure 1). These patients were considered seropositive for *T. canis* IgG antibodies because of both weak LMW bands and absence of other helminth eggs in repeated feces microscopy.

We have detected *T. canis* seropositivity in 17.8% of 73 patients with CU. It is also affected by socio-demographic variables such as age, sex, education level, monthly income, daily hand washing habit, soil-eating and dog feeding history. These data suggest that encountering the patients with T. canis may trigger CU. We believe that further studies with more patients in this subject will be helpful to demonstrate the role of Toxocara on ethiopathogenesis of CU.

REFERENCES

1. Despommier D. Toxocariasis: Clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev 2003; 16(2):265-72.
2. Aminoff MJ, Boller F, Swaap DF. Handbook of Clinical Neurology. Garcia H, Tanowitz H, Del Brutto O. Neuroparasitology and Tropical Neurology, 1st Edition. In: Nicoletti A. Toxocariasis. USA, Elsevier, Volume 114, 2013: 217–228.
3. Magnaval JF, Glickman LT, Dorchies P, Morassin B. Highlights of human toxocariasis. Korean J Parasitol 2001; 39(1):1-11.
4. Overgaauw PA. Aspects of Toxocara epidemiology: Human toxocarosis. Crit Rev Microbiol 1997; 23(3):215-31.
5. Charlesworth EN. CU: Background, evaluation, and treatment. Curr Allergy Asthma Rep 2001; 1(4):342-7.
6. Demirci M, Yildirim M, Aridogan BC, Baysal V, Korkmaz M. Tissue parasites in patients with CU. J Dermatol 2003; 30(11):777-81.
7. Giacometti A, Cirioni O, Fortuna M, et al. Environmental and serological evidence for the presence of toxocariasis in the urban area of Ancona, Italy. Eur J Epidemiol 2000; 16(11):1023-6.
8. Mizgajska H. Eggs of Toxocara spp. in the environment and their public health implications. J Helminthol 2001; 75(2):147-51.
9. Oge S, Oge H. Prevalence of Toxocara spp. eggs in the soil of public parks in Ankara, Turkey. Dtsch Tierarztl Wochenschr 2000; 107(2):72-5.
10. Magnaval JF, Fabre R, Maurieres P, Charlet, J.P, de Larrard B. Application of the western blotting procedure for the immunodiagnosis of human toxocariasis. Parasitol Res 1991; 77(8):697-702.
11. Zwolinski J. The risk factors of Toxocara canis infestation in population of patients from the Lublin Region. Wiad Parazytol 2000; 46(4):463-73.
12. Humbert P, Niezborala M, Salembier R, Aubin F, Piarroux R, Buchet S, et al. Skin manifestations associated with toxocariasis: A case-control study. Dermatology 2000; 201(3):230-4.
13. Gavignet B, Piarroux R, Aubin F, Millon L, Humbert P. Cutaneous manifestations of human toxocariasis. J Am Acad Dermatol 2008; 59(6):1031-42.
14. Cassenote AJ, Lima AR, Pinto Neto JM, Rubinsky-Elefant G. Seroprevalence and Modifiable Risk Factors for Toxocara spp. in Brazilian Schoolchildren PLoS Negl Trop Dis. 2014 May; 8(5): e2830.
15. Yazar S, Yaman O, Cetinkaya U, Hamamci B, Sahin I. Investigation of Anti-Toxocara canis IgG antibodies in patients presenting at the Erciyes University Medical Faculty, Department of Parasitology. Turkiye Parazitol Derg 2010; 34(1):24-6.
16. Wolfrom E, Chene G, Lejoly-Boisseau H, Beylot C, Geniaux M, Taieb A. CU and Toxocara canis infection: a case control study. Ann Dermatol Venereol 1996; 123(4):240-6.
17. Chiodo P, Basualdo J, Ciarmela L, Pezzani B, Apezteguía M, Minvielle M. Related factors to human toxocariasis in a rural community of Argentina. Mem Inst Oswaldo Cruz 2006; 101(4):397-400.
18. Roldan WH, Espinoza YA, Huapaya PE, Huiza AF, Sevilla CR, Jiménez S. Frequency of human toxocariasis in a rural population from Cajamarca, Peru determıned by DOT-ELISA Test. Rev Inst Med Trop Sao Paulo 2009; 51(2):67-71.

19. Romano N, Nor Azah MO, Rahmah N, Lim Y AL, Rohela M. Seroprevalence of toxocariasis among Orang Asli (Indigenous people) in Malaysia using two immunoassays. Trop Biomed 2010; 27(3):585-94.
20. Stensvold CR, Skov J, Moller LN, Jensen PM, Kapel CM, Petersen E et al. Seroprevalence of human toxocariasis in Denmark. Clin Vaccine Immunol 2009; 16(9):1372-3.
21. Won KY, Kruszon-Moran D, Schantz PM, Jones JL. National seroprevalence and risk factors for zoonotic Toxocara spp. infection. Am J Trop Med Hyg 2008; 79(4):552-7
22. Demirci M, Korkmaz M, Sakru N, Kaya S, Kuman A. Diagnostic importance of serological methods and eosinophilia in tissue parasites. J Health Popul Nutr 2002; 20(4):352-5.
23. Rubinsky-Elefant G, da Silva-Nunes M, Malafronte RS, Muniz PT, Ferreira MU. Human toxocariasis in rural Brazilian Amazonia: Seroprevalence, risk factors, and spatial distribution. Am J Trop Med Hyg 2008; 79(1):93-8.
24. Santarem VA, Leli FN, Rubinsky-Elefant G, Giuffrida R. Protective and risk factors for toxocariasis in children from two different social classes of Brazil. Rev Inst Med Trop Sao Paulo 2011; 53(2):66-72.
25. Doğan N, Dinleyici EC, Bor O, Töz SO, Ozbel Y. Seroepidemiological survey for Toxocara canis infection in the northwestern part of Turkey. Turkiye Parazitol Derg 2007; 31(4):288-91.
26. Thompson DE, Bundy DA, Cooper ES, Schantz PM. Epidemiological characteristics of Toxocara canis zoonotic infection of children in a caribbean community. Bull World Health Organ 1986; 64(2):283-90.
27. Karadam SY, Ertug S, Ertabaklar H, Okyay P. The comparison of IgG antibodies specific to Toxocara spp. among eosinophilic and non-eosinophilic groups. New Microbiol 2008; 31(1):113-6.
28. Glickman LT, Magnaval JF, Domanski LM, Shofer FS, Lauria SS, Gottstein B, et al. Visseral larva migrans in French adults: A new disease syndrome. Am J Epidemiol 1987; 125(6):1019-34.
29. Maraghi S, Rafiei A, Hajihossein R, Sadjjadi SM. Seroprevalence of toxocariasis in hypereosinophilic individuals in Ahwaz, South-Western Iran. J Helminthol 2012; 86(2):241-4.
30. Yamasaki H, Araki K, Lim PK, Zasmy N, Mak JW, Taib R, et al. Development of a highly specific recombinant Toxocara canis second-stage larva excretory-secretory antigen for immunodiagnosis of human toxocariasis. J Clin Microbiol 2000; 38(4):1409-13.
31. Özkoç S, Bayram Delibaş S, Akısü Ç. Toksokariyazis serolojik tanısında Trichinella çapraz reaksiyonlarının degerlendirilmesi. Mikrobiyol Bul 2012; 46(3):456-63.