

# Seroprevalence of *Toxocara spp* Among Epileptic Patients in Iraq/Basra

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**Abstract:** To study seroprevalence of *Toxocara* antibody Immunoglobulin G (IgG) and epidemiological risk factors as well as assessment the immunological pattern including cytokines, interleukin-6 (IL6) and interleukin-10 (IL-10) and immunoglobulin E (IgE) among epileptic patients in Basra province . **Subjects and Methods:** Forty seven epileptic patients, their ages range from 2.5 - 45 years (22 females and 25 males) and 109 apparently healthy volunteers as a control group with ages range between 2 - 80 years (59 females and 50 males ) were enrolled in this study. The study was done during the period from December 2017 to November 2018 at The Pediatric and Neurology Consulting Clinic of Basra Teaching Hospital. The risk and epidemiologic factors were assessed by a special questionnaire which was completed by the patients themselves or their relative. Immunoglobulin G (IgG) and cytokines were assessed by using ELISA while IgE by immunoturbidimetric assay by using the Abbott ARCHITECT c System for every epileptic patient as well as control group. **Results:** Sixteen (34.04%) of epileptic patients were seropositive for *Toxocara* antibody (IgG ) in comparison with 2 (1.83%) of control group with significant difference between them ( $p=0.0001$ ). There was no significant association between seropositive and seronegative epileptic patients in regard to characteristic features of the studied population (sex and age) and risk factors (residence, exposure to soil, animal ownership, presence of home garden, onychophagia, thumb sucking, medicine intake, family history, duration of epilepsy and occupation) except geophagia . But interestingly, there were elevated in risk ratio for 5 variables including onychophagia (1.7), thumb sucking (2.1), geophagia (3.4), medication intake (1.1) and family history (1.7), when it is evaluated by Chi-squared "Fisher exact test" (risk ratio >1). The immunological assessments reveal an elevation IgE and IL-10 level in seropositive group in comparison seronegative group without a significant difference, while IL-6 concentration is elevated in the seronegative group. **Conclusion:** In epileptic patients in Basra province, infection with *Toxocara spp* may play role as a risk factor for idiopathic epilepsy. Risk factors such as onychophagia, geophagia, thumb sucking, medication intake and family history may increase risk infection with *Toxocara spp* in epileptic patients. Also toxocariasis may lead to elevation of IgE and IL-10 levels in these patients but it appears has a weak influence on concentration of IL-6.

**Keywords:** *Toxocara spp* ;Neurotoxocariasis ;Epilepsy; IL-10 ;IL-6

## 1. INTRODUCTION

Toxocariasis is caused by nematodes(roundworm) belong to the genus *Toxocara*, which is consist of more than 30 species[21]. Human toxocarasis caused by *Toxocara canis* and *Toxocara cati* [22]. *Toxocara canis* is the nematode most frequently of Canidae [5], and *Toxocara cati* for cats [18], while other animal species represent a reservoir for these nematodes such as rats, birds, while human represent an accidental host[5]. These parasites can infect other paratenic hosts like invertebrates [24]. *T. canis* and *T. cati* like other Soil-transmitted nematodes are neglected in the international public health importance when they are comparing with other helminthic diseases [22].

The symptomatic toxocariasis gives 3 clinical features including ocular larva migrans (OLM), visceral larva migrans (VLM) and neurological toxocariasis(NT) [17].

While covert or common toxocariasis is a subclinical form of *Toxocara* infection [23].

Neurotoxocariasis (NT) can be defined as manifestation of *Toxocara* infection when *Toxocara* larvae reach to CNS for invading the brain and spinal cord. Neurotoxocariasis may be influenced by several factors, like number of swallowed ova, host genetic factors and previously exposure to infection [37,15] and it is rare which mainly attack people with middle-aged. (NT) can lead to induction of meningitis, encephalitis, myelitis and cerebral vasculitis including also relatively non-specific clinical symptoms such as fever and headache [16,8,11]. Epilepsy is considered as one of the clinical manifestations of NT [16].

Early researchers have found that epileptic patients are highly exposure to infection with *T.canis* [36,9]. Other researchers work on numerous populations and communities for studying the correlation between toxocariasis and epilepsy found the possibility of

consideration the toxocariasis as a cofactor for epilepsy occurrence especially in endemic areas [6,7, 39].

This is the first study on toxocariasis among epileptic patients in Basra Province, which designated to record *Toxocara spp* seroprevalence and to assess the immunology among epileptic patients in comparison to apparently healthy persons.

**Subjects and Methods.**

**Studied population:**

This study was carried out at Basra Teaching Hospital from December 2017 to November 2018, on 47 epileptic patients (25 males and 22 females). Their ages range between 2.5 - 45 years attending Pediatric and Neurology Consulting Clinic at Basra Teaching Hospital. Patients with history of diabetic mellitus, rheumatic diseases, diarrhea and abdominal pain were excluded from the study. In addition, 109 apparently healthy persons were volunteered as a control ,their ages range between 2 - 80 years. Epileptic patients underwent a thorough clinical examination. Cranial imaging studies (CT scanning and MRI) were done to all patients in addition to standard EEG recording, blood investigation in order to confirm diagnosis.

**Questionnaire:**

A questionnaire was applied to all patients and controls to obtain socioeconomic and epidemiological information e.g. sex, age, animal ownership, presence of garden at home, exposure to soil, onychophagia or geophagia habit, thumb sucking, occupation, medicine intake, family history of the disease, residence, and duration of disease.

**Collection of samples:**

five milliliter blood was taken from every patients and controls under aseptic conditions by plane tube with gel and clot activator without EDTA. Sera was later separated from clotted blood by centrifugation and immediately frozen at -80°C until used.

**Measurement of seropositivity for toxocariasis:**

*Toxocara* antibodies were detected by the commercial human IgG *Toxocara* microwell serum and plasma ELISA Kits (T8072,usbiological life science, united states)with sensitivity 87.5% and specificity 93.3% According to the protocol, the result is positive when the absorbance reading  $\geq 0.3$  OD units while the negative  $< 0.3$  OD unites.

**Measurement of total IgE in serum:**

Serum IgE levels were evaluated in all seropositive group against 16 of seronegative group and 30 of control taken by using kits QUANTIA IgE Reagent Kit (6k42-01) (biokit S.A. Can Male, s/n08186 Llica d Amunt Barcelona Spain distributed by Abbott ). This kit intended for quantitative determination of IgE (IU/mL) in plasma or serum by immunoturbidometric assay by using the Abbott ARCHITECT c System using standard protocol as mentioned in the kit through using Architect c4000 system appature (Japan).

**Measurement of IL-10 and IL-6 concentration:**

The same number of serum samples for each group and method of preparation of serum sample used for measurement of total IgE have been used to detect concentration of IL-10 and IL-6 by using Human IL-10 ELISA kit (E-EL-H0103) and Human IL-6 ELISA kit (E-EL-H0102) from Elbscience (USA). After following each one procedure, the optical density (OD value) was determined by micro –plate reader set to 450 nm and then calculating the concentration according to kite procedure.

**Data analysis:**

Data were analyzed by using (ANOVA) which is supported by Turkey 's spost . Relative risk ratio was evaluated by Chi–squared “Fisher exact testes” (risk ratio>1) using Koopman asymptomatic score . The significant p-value is at  $p<0.05$ . Data analysis was performed by GraphPad Prism software for windows (version 7, GraphPad Software, Inc).

**Results:**

Sixteen out of 47 (34.04%) samples analyzed were positive for *Toxocara* antibodies in comparison to 2 (1.83%) positive samples of control (both were 40 years old). So the difference was significant between epileptic patients and control(apparently health) group ( $p=0.0001$ ) as shown in table-1. Table-2 show absence the significant correlation between seropositive epileptic patients &seronegative epileptic patients ( $p=0.99, 0.18$  respectively) in relation to sex and age.

Table-1: Seroprevalence of Toxocarasis of epileptic patients

Groups	Epilepsy Toxocara Positive Total no. = 16	Epilepsy Toxocara Negative Total no. = 31	Control Group Total no. = 109	P-value
Seropositive	16 (34.04%)	-----	2 (1.83%)	0.0001
seronegative	-----	31 (65.96%)	107 (98.17%)	

Table-2: Characteristic features of epileptic patients

variable	Seropositive epileptic patients No=16	Seronegative epileptic patients No=31	p-value
Sex Male female	9 7	16 15	o.99
Age groups(years) 2.5-18 19-39 40-45	12 4 4 4	17 9 5 3	0.18

Table-3 shows no significant correlation between *Toxocara* prevalence rate and risk factors (residence , ,exposure to soil, animal ownership, presence of home garden,

onychophagia, thumb sucking, medicine intake, family history, duration of epilepsy disease and occupation) between seropositive and seronegative epileptic patients. However, geophagia shows a significant deference ( $p=0.004$ ). Also Table -3 shows, according to statistical analysis by Chi-squared (Fisher exact test; risk ratio $>1$ ), an increase in risk ratio of five variables risk factors (onychophagia, thumb sucking, geophagia, medication intake and family history (risk factor=1.7, 2.1, 3.4, 1.1, 1.7 respectively) in contrast to other variable factors regarding their influences on seroprevalence of *Toxocara* antibody in epileptic patients.

Table-3: Risk factors for toxocariasis in epileptic patients

Variables	Seropositive epileptic patients No=16	Seronegative epileptic patients No=31	p-value	Risk factor
Residency City center uptown	10 6	23 8	0.506	0.85
Exposure to soil High Low	8 8	21 10	0.343	0.62
Geophagia Yes No	6 10	1 30	0.004	3.4
Animals ownership (dog or cat) Yes no	6 10	11 20	0.99	1
Garden in house Yes no	7 9	15 16	0.99	0.8
Onychophagia Yes No	6 10	6 25	0.28	1.7
Sucking thumb Yes No	2 14	1 30	0.26	2.1
Medication intake Yes No	15 1	28 3	0.07	1.1
Family history Yes No	9 7	11 20	0.336	1.7
Duration Less than 1 year 1-3years 4-5 years More than 5 years	3 3 5 5	9 5 3 14	0.276	

Fig-1: Concentration level of IgE in patients and control groups.

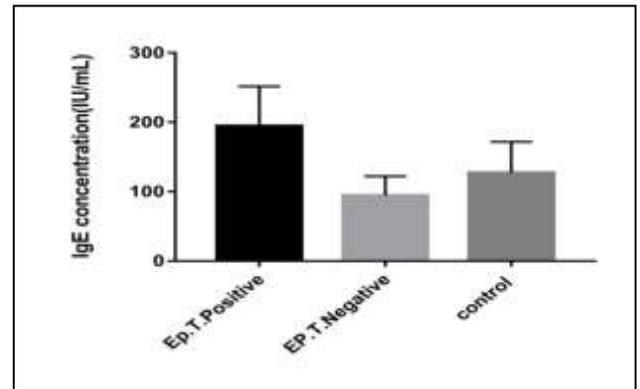


Fig-1 show that levels of IgE (total concentration IU/mL) in the serum of epileptic patients with seropositive *Toxocara* antibody (Ep.T. positive) are higher when it compared with seronegative (Ep.T. negative) and control group, but there is no significant difference among these groups .

Fig-2 Concentration level of IL-10 in patients and control groups.

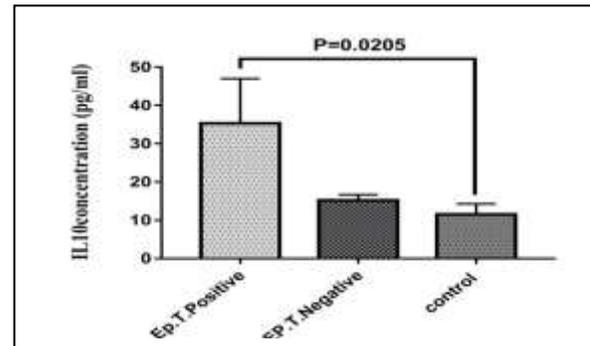
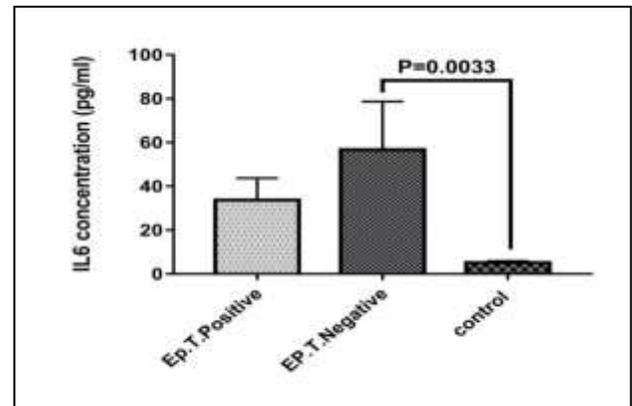


Fig-3 Concentration level of IL-6 in patients and control groups



As shown in Fig-2 the epileptic patients with seropositive *Toxocara* antibody have increased levels of

IL-10 (concentration pg/ml) than seronegative patients (Ep.T. negative) and control group. While there was a significant correlation between seropositive group and control. ( $p=0.0205$ ). Furthermore, there was elevation in the levels of IL-6 in serum of seronegative with a significant with control group ( $P=0.0033$ ) as shown in Figure-3.

### Discussion

*Toxocara* infection is prevalent among stray dog in Basra province. Findings of [1] suggested that 26.5% of stray dogs in different regions of Basra were infected with *Toxocara canis*. The present study on epileptic patients has revealed an elevated titer of *Toxocara spp* antibody in those patients with significant difference as compared with control group. These results agree with previous studies of [25, 26, 39, 4] in rural Bolivia, Italy and Iran respectively. There were several mechanisms for explaining the mechanism of epileptogenesis of *Toxocara* infection like, Larvae of *Toxocara* could be surrounded by granuloma which can lead to acute symptomatic seizures. Then may leave fibrous scars and chronic granulomatous lesions after resolution which may cause epilepsy [34] However, others didn't find significant correlation between epilepsy and *Toxocara* seropositivity such as [13,14] in Egypt.

One of present findings is absence significant correlation regarding characteristic features of population (sex and age) with *Toxocara* infection and this is in agreement with findings of [32] in Egypt, while [13] found increasing rate of toxocariasis among epileptic males.

Present study hasn't shown significant effect of residency regarding *Toxocara* seropositivity in accordance with findings of [39] and also it doesn't represent a risk factor for infection and this is may be explained by exposure of epileptic patients to the same predisposing factors for infection with toxocariasis. In contrast with findings of [13] who found a significant relationship between rural areas and *Toxocara* infection.

There wasn't significant association between seropositivity of *Toxocara* antibody and pets breeding (dogs or cats) in the present study, the same was found by [26]. Also pets, in present study, don't seem to be a risky for infection and this may be due to few of people keeping animals, mainly dogs, in their houses especially in city center, and this is in disagreement with [35] and [1] who found that dog ownership increases the risk of exposure to infection with *Toxocara spp*.

Other present finding is absence significant relationship between the exposure to soil and infection with *Toxocara* which is in agreement with result of [32] in Egypt Also presence of garden in patient's house isn't significant which is similar to the findings of [3] in Turkey. On the other hand, geophagia in this study shows increased risk for toxocariasis with a significant value which is the same finding of [6]. Because The main mode of transmission to

toxocariasis is swallowing of infective eggs which contaminate the soil [29], so geophagia facilitates the infective eggs to reach the mouth. These findings don't match with what was found by [3]. This study proves the elevation of risk ratio of onychophagia and thumb sucking for *Toxocara* infection as previously researched [31, 2] as these habits may increase infection by facilitate reaching of infective eggs to mouth.

Medication intake and different types of medicines appear to be a risk factor for toxocariasis in the present work. This can be explained by the effects of antiepileptic drugs on the immune system as hypersensitivity or immune suppression [19].

Patients who have family history of epilepsy would have a high risk of being *Toxocara* seropositive as compared to other with no family history that may be explained by the genetics of the host that determine the resistance or susceptibility to parasitic infection via innate or acquired immunity [30].

[35] found that lower socioeconomic status can be correlated with occupations that increase exposure risk to toxocariasis, in contrast with the findings of the present work which indicates that there is no correlation between seropositivity and socioeconomic status. This is because all patients in this study are nearly at the same socioeconomic status.

The best of our knowledge, there is no published article on the correlation between *Toxocara*, epilepsy and immunology to compare with it.

The elevation in level of total IgE in serum of seropositive epileptic group that is shown in this work can be explained by that the parasitic antigens stimulate Th2 cells that leads to stimulate cytokines production such as interleukin 4 &5 which induce IgE production [28].

The present work shows elevation level of IL-10 concentration in seropositive epileptic patients than in others with seronegative or control groups with a significant difference between them and control. Fig- 2.

This elevation can be attributed to the results of [20] that IL-10 is up regulated in mice when *Toxocara* larvae reached to their brains. Furthermore, it has been noticed elevation in level of IL-10 in plasma after beginning of seizure for 48-72 hours which reflects a protective role for this cytokine as an anticonvulsive factor that suppresses the proinflammatory cytokine production [38].

Also, through the present study it appears that the group who is seropositive to *Toxocara* with elevation concentration level of IL-10 has low concentration level of IL-6 (Figure-3) which agrees with the fact that IL-10 may have a role in decreasing synthesis of nonspecific proinflammatory cytokines such as IL-6 [10]. Also previous experiment on splenocytes from infected mice with *Toxocara* has confirmed the production of less amounts of IL-6 as compared to control group [12], but [27] has found elevation in IL-6 mRNA level of mice infected with *Toxocara*, and this could be related to a possible role of IL-

6 in the course of neurotoxocariasis mainly in the chronic stage. Interleukin-6 concentration is found to be elevated in seronegative group in the present study which is similar to the findings of the study of [33] which shows increased IL-6 concentrations in epilepsy patients without toxocariasis as compared with healthy controls.

**Conclusion:** In idiopathic epileptic patients in Basra province, infection with *Toxocara spp* may play a role as a risk factor for epilepsy. Risk factors such as geophagia, onychophagia, thumb sucking, medication intake and family history may increase infection risk with *Toxocara spp* in epileptic patients. Also toxocariasis may leads to elevation in the concentrations of IgE and IL-10 in these patients but it appears to have a weak influence on the concentration of IL-6.

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