

Seropositivity of *Toxoplasma gondii* and *Toxocara* spp. in Children with Cryptogenic Epilepsy, Benha, Egypt

Maysa Ahmad Eraky^{1*}, Soha Abdel-Hady², Karim Fetouh Abdallah³

¹Parasitology Department, Faculty of Medicine, Benha University, Benha 13518, Egypt; ²Pediatrics Department, Faculty of Medicine, Benha University, Benha 13518, Egypt; ³Medical Parasitology Department, College of Medicine, Taif University, K.S.A

Abstract: The present study aimed to investigate the possible association of *Toxoplasma gondii* and *Toxocara* spp. infections with cryptogenic epilepsy in children. The study was carried out between June 2014 and March 2015. Total 90 children (40 with cryptogenic epilepsy, 30 with non-cryptogenic epilepsy, and 20 healthy control children) were evaluated to determine the anti-*Toxocara* and anti-*T. gondii* IgG seropositivity using ELISA kits. Epileptic cases were selected from those attending the pediatrics outpatient clinic of Benha University Hospital, Pediatrics Neurology Unit, and from Benha Specialized Hospital of children. The results showed that the level of anti-*T. gondii* IgG seropositivity was significantly higher among children with cryptogenic epilepsy (20%) than among children with non-cryptogenic children (0%). In healthy controls (10%), there was no association between toxocariasis seropositivity and cryptogenic epilepsy (only 5.7%; 4 out of 70 cases) among cases and 10% (2 out of 20) among controls. Among toxocariasis IgG positive cases, 3 (7.5%) were cryptogenic, and only 1 (3.3%) was non-cryptogenic. These statistically significant results support the association between *T. gondii* infection and cryptogenic epilepsy while deny this association with toxocariasis.

Key words: *Toxoplasma*, *Toxocara*, child, cryptogenic epilepsy, seropositivity, Egypt

Epilepsy is a group of neurological disorders characterized by epileptic seizures [1,2]. Two types of epilepsy are known: idiopathic epilepsy with unknown etiology and the secondary epilepsy which originates from cerebral lesions that may be traumatic, hypoxic, or infectious in origin [3,4]. In about 60% of cases, the cause is unknown [5]. Epilepsy is affecting about 1% of people worldwide (65 million) [6]. Another report indicates that 8.5 per 1,000 persons have epilepsy [7,8], and the prevalence of the disease is higher in poor countries and nearly 80% of cases occur in developing countries [9]. In high-income countries, traumatic head/brain injuries and stroke are the main causes of epilepsy [10,11], whereas in low- and middle-income countries, central nervous system infections due to viral, bacterial, and parasitic infections seem to contribute to the high prevalence of epilepsy [7]. Epidemiological studies suggest helminthic infections in association with epilepsy in low income countries of the world. *Toxoplasma* infections are

implicated to have an association with epilepsy either as a cause or a potential risk factor [12].

Toxocara spp. is one of the common helminthic parasites that can affect human central nervous system (CNS). Several studies have reported an association between *Toxocara canis* infection and epilepsy in different geographic locations through case-control studies using serological tests [13-15]. The diagnosis of human toxocariasis depends on serological test (ELISA) by using excretory-secretory antigens from *Toxocara* larvae because it is very difficult to detect infective *Toxocara* larvae in biopsy specimens. Until present, there is no precise report on anti-*Toxoplasma* and anti-*Toxocara* antibodies in epileptic children in Benha City, Egypt. Therefore, the objective of the present investigation was to examine the antibody response to *T. gondii* and *Toxocara* infections in epileptic children using ELISA test.

The current study was carried out between June 2014 and March 2015. The study was approved by the Ethical Committee of Faculty of Medicine, Benha University, Egypt in 2014. The purpose and procedures of the study were explained and written informed consent was obtained from all parents or legal guardians of the children participating in the study on behalf of all child participants. Total 90 children aged between 9 months to 18 years were enrolled (Table 1). Children were di-

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*Corresponding author (maysa.hassan@fmed.bu.edu.eg; erakymaysa@yahoo.com)

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Table 1. Sociodemographic data of studied groups

		Epileptic (%)		Non-epileptic (%)	P-value
		Cryptogenic	Non-cryptogenic		
Age/year (\pm SD)		4.36 \pm 2.95	7.13 \pm 3.23	4.62 \pm 2.43	<0.00 ^a
Sex	Male	19 (47.5)	14 (46.7)	9 (45.0)	0.92 ^b
	Female	21 (52.5)	16 (53.3)	11 (55.0)	
Residence	Urban	14 (35.0)	11 (36.7)	8 (40.0)	0.93 ^b
	Rural	26 (65.0) ^c	19 (63.3)	12 (60.0)	
Social class	Low	23 (57.5)	18 (60.0)	13 (65.0)	0.81 ^b
	Moderate	13 (32.5)	10 (33.3)	5 (25.0)	
	High	4 (10.0)	2 (6.7)	2 (10.0)	

^aSignificant difference between cryptogenic group and the other 2 groups.

^b χ^2 -test was used to compare between the 3 investigated groups.

^cSignificant difference between positives and negatives within each group (using Z-test).

vided into 3 groups (G1, G2, and G3). Group1 (G1) composed of 40 cryptogenic epileptic children presented with recurrent epileptic fits with unknown etiology. All selected patients had no past history of head trauma, brain surgery, previous meningitis, encephalitis, with normal brain Magnetic Resonance Imaging scan (MRI), and no family history of epilepsy. The second group (G2) composed of 30 epileptic children presented with recurrent epileptic seizures with known causes as head trauma, family history of epilepsy, brain surgery, previous encephalitis, or meningitis. The third group (G3) composed of 20 completely healthy volunteer children. The epileptic cases were selected from those attending the pediatrics outpatient clinic of Benha University Hospital, pediatrics neurology unit, and from Benha Specialized Hospital of children. Three-ml of blood was taken from all children, centrifuged at 1,000 rpm, and the sera were stored at -20°C until use.

Anti-*T. gondii* IgG antibody levels were determined using commercially available quantitative ELISA kit, i.e., DRG[®] *Toxoplasma* IgG (TORCH) Catalog No. EIA-1798 (DRG International, Inc., Mountainside, New Jersey, USA). The test was performed following the manufacture's guidance. In brief, 100 μ l of each diluted serum samples (1:40) was added to *T. gondii* antigen coated microtiter wells. Following incubation for 30 min, 100 μ l of 1:1,000 diluted horseradish peroxidase-conjugated anti-human IgG was added. After a second incubation, tetramethylbenzidine (TMB) substrate was then added to each well to stop solution. The optical density (OD) values were read at 450 nm using an automated microplate reader.

Serum samples were also analyzed for anti-*T. canis* IgG antibodies by a commercially available quantitative ELISA using RIDASCREEN *Toxocara*-IgG ELISA (R-Biopharm AG, Darm-

stadt, Germany) kit which detects antibodies against the excretory-secretory antigen of *Toxocara* larvae. The test was performed following the manufacturer's instructions.

The data were analyzed statistically using SPSS version 16 software (SPSS Inc., Chicago, Illinois, USA). Categorical data were presented as number and percentages. Chi-square test, Fisher's test, and Student's *t*-test were used as tests of significance. The values of $P < 0.05$ were considered to be statistically significant.

Gender differences with epilepsy were not clear. There is broad agreement between studies that females have a marginally lower incidence of epilepsy and unprovoked seizures than males. In the present study, epilepsy was slightly higher in females (52.5% of cryptogenic and 53.3% of non-cryptogenic epileptics) vs 47.5% of cryptogenic and 46.7% of non-cryptogenic epileptic males [16]. In our study, 64.3% of epileptic groups were residing in rural areas and 35.7% were residing in urban areas. This significant difference indicates that epilepsy is more common in rural areas, which doesn't match with what was recorded by El-Tantawy et al. [12] who reported no significant relations between epilepsy and residence. Cerebral toxoplasmosis has been reported to cause seizures in about 25% of infected cases [16] by producing diffuse encephalitis or localized lesions [17].

In the present study, there was a significant association of *T. gondii* exposure and cryptogenic epilepsy, as 20% of epileptic cryptogenic group were seropositive compared to 10% of the controls ($P = 0.017$) (Table 2). Thus, latent toxoplasmosis may be an underlying cause for cryptogenic epilepsy [13,18].

This correlation can be explained by either the presence of dormant *T. gondii* cysts that can cause epileptic foci. Some of

Table 2. Anti-*Toxoplasma* and anti-*Toxocara* seropositivity among epileptic and non-epileptic children

IgG seropositive No. (%)		Epileptic (%)		Non-epileptic (%)	P-value
		Cryptogenic	Non cryptogenic		
<i>Toxoplasma</i>	Positive	8 (20.0)	0 (0)	2 (10.0)	0.017 ^{a,b}
	Negative	32 (80.0) ^c	30 (100)	18 (90.0) ^c	
<i>Toxocara</i>	Positive	3 (7.5)	1 (3.3)	2 (10.0)	0.66 ^b
	Negative	37 (92.5) ^c	29 (96.7) ^c	18 (90.0) ^c	

^aSignificant difference between cryptogenic group and the other 2 groups.

^b χ^2 -test was used to compare between the 3 investigated groups.

^cSignificant difference between positives and negatives within each group (using Z-test).

Table 3. Sociodemographic data for anti-*Toxoplasma* seropositive epileptic cases

		No. (%) of cryptogenic anti- <i>Toxoplasma</i> seropositive cases	P-value
Sex	Male	6 (75.0)	0.1
	Female	2 (25.0)	
Residence	Urban	1 (12.5)	<0.001 ^a
	Rural	7 (87.5)	
Social class	Low	5 (62.5)	0.26
	Moderate	3 (37.5)	
Animal contact	Yes	5 (62.5)	0.26
	No	3 (37.5)	
Convulsion	Localized	1 (12.5)	<0.001 ^a
	Generalized	7 (87.5)	

^aSignificant difference between cryptogenic group and the other 2 groups.

the tissue cysts may rupture and cause marked inflammation that can trigger microglial formation which may represent the 'tombstones' of *Toxoplasma* cysts and end to scarring [19]. The cryptogenic epilepsy patients could be also more susceptible than others to such infections for reasons unrelated to epilepsy, or due to intrinsic immunologic differences that predispose them to epilepsy [13,20]. On the other hand, no relationship was reported between cryptogenic epilepsy and *T. gondii* IgG seropositivity [21].

Our findings demonstrated that *Toxoplasma* seropositivity was more common in males (75%) than in females (25%), as males are more frequently infected than females in most endemic areas [22], especially those from rural (87.5%) areas with low social class (62.5%) [12]. Another important risk factor associated with *Toxoplasma* infection is animal contact (Table 3). In this work, there was significant correlation between *Toxoplasma* infection and positive history of contact with animals (mainly cats and dogs), as 5 out of the 8 anti-*Toxoplasma* seropositive cases (62.6%) gave positive history for contact

with animals.

The association between toxocariasis and epilepsy is well documented. However, few data are available concerning the relationship between *T. canis* infection and epilepsy in Egypt. Our results did not observe any association between toxocariasis and epilepsy, as there was no statistically significant difference between epileptic cases and healthy controls ($P > 0.6$). Anti-*Toxocara* IgG antibodies were found in 4 epileptic children (3 in cryptogenic and only 1 in non-cryptogenic groups), and 2 seropositive cases were found in control subjects.

Similar findings were reported by El-Tantawy et al. [12] who found no association between *Toxocara* seropositivity and epilepsy, as 64 (48.5%) of children with cryptogenic epilepsy have positive anti-*Toxocara* antibodies in comparison to controls, 28 (46.7%). Arpino et al. [23] reported an association between anti-*Toxocara* antibody titers and seizures and suggested that toxocariasis might have a role as a cofactor in epileptic seizures. Many other case-control studies have been carried out in different locations to investigate the possible association between *T. canis* seropositivity and epilepsy. A positive and significant association has been reported in Bolivia, Burundi, UK, Italy, and USA [14,15,20,24,25]. The difference in visceral larva migrans types as well as the differences in diagnosis practices across tropical regions may help to explain the differences in the prevalence of epilepsy resulting from toxocariasis [26].

Based on the data of the current study, toxoplasmosis should be considered as a possible epilepsy risk factor, as there was a significant association between *T. gondii* seropositivity and cryptogenic epilepsy.

CONFLICT OF INTEREST

The authors declare that no competing interest exists in this study.

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