

Seroprevalence of *Toxoplasma Gondii* and *Toxocara Spp* in Children with Cryptogenic Epilepsy

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Abstract Cryptogenic epilepsy is a group of epilepsy syndromes where aetiology is unknown but an underlying brain disease is suspected. Increased seropositivity for *Toxocara* and *Toxoplasma gondii* have been observed in epileptic patients with sparse data about their seropositivity in cryptogenic epileptic patients. Therefore, we investigated the probable relationship between seropositivity against *T. gondii* and *Toxocara* with cryptogenic epilepsy. We examined patients who had cryptogenic epilepsy and healthy non epileptic controls for seropositivity for *Toxocara* and *T. gondii* antibodies by ELISA. Out of 132 cryptogenic epileptic patients, 80 (60.6 %) and 64 (48.5%) were seropositive for *T. gondii* and *Toxocara* immunoglobulin G (IgG) antibodies respectively. The seropositivity in the control group was 26 (43.3%) and 28 (46.7%) for *T. gondii* and *Toxocara* IgG respectively. We found a significant association between chronic *T. gondii* infection and cryptogenic epilepsy while the association between *Toxocara* infection and cryptogenic epilepsy was insignificant. Our findings indicate that toxoplasmosis may be a cause of cryptogenic epilepsy. We recommended both promoting health education to prevent such infection and screening children for toxoplasmosis which would help early treatment and so decreasing the incidence of epilepsy.

Keywords: *Toxoplasma*, *Toxocara*, cryptogenic epilepsy, ELISA

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1. Introduction

Epilepsy is a chronic neurological disorder that affects approximately 70 million people of all ages worldwide [1]. Nearly 80% of people with epilepsy are found in developing countries, where it remains a major public health problem, not only because of its health implications but also for its social, cultural, psychological and economic connotations [2]. According to the Commission on Classification and Terminology of the International League Against Epilepsy in 1985-1989 [3], cryptogenic epilepsies are defined as epilepsy syndromes for which an aetiology is unknown, but an underlying brain disease is suspected affecting approximately 20% of all patients with epilepsy [4].

Parasitic infections are considered as serious public health problems in many developing countries [5] affecting various tissues including the brain leading to neurological dysfunction and constitute important risk factors for epilepsies [6]. Simultaneously, these infections represent a controllable risk as they can be prevented through immunization or treatment. Among these parasitic infections, toxoplasmosis and toxocariasis have been

suggested as an important risk factor for epilepsy needing confirmation [7,8].

Toxoplasmosis is caused by *Toxoplasma gondii*, a protozoan and is estimated to infect one-third of the world's human population [9,10]. *Toxoplasma* is an obligate intracellular parasite founded in two forms in humans. The actively proliferating trophozoites are usually seen in the early phases of the infection while the resting forms or tissue cysts are primarily found in muscle and brain [11]. Cerebral toxoplasmosis has been reported to cause seizures in about 25% of infected cases [12] by producing diffuse encephalitis or localized lesions [13].

Toxocariasis is a zoonosis caused by *Toxocara canis* and *T. cati*, the nematode parasites of dogs and cats, respectively [14]. The principal source of infection is eggs which are released from the feces of dogs and result in contamination of the soil [15,16]. Children are more susceptible to this infection because they handle (playing habits) contaminated soil [17]. When infective eggs containing larvae are ingested by human, the larvae become free in the intestine and pass through the intestinal wall to migrate through tissues provoking inflammatory tissue reactions with multisystem involvement leading to the clinical syndrome referred to as visceral larva migrans [18]. The larvae can locate in the central nervous system

leading to a variety of neurological disorders [19]. Seizures have been reported as the initial CNS manifestation of toxocariasis in quite a number of patients [20]. A possible association between *Toxocara* infection and epilepsy has been hypothesized and toxocariasis has been suggested as a cofactor for epilepsy [21].

Both *Toxoplasma* and *Toxocara* infections are implicated to have an association with epilepsy either as a cause or a potential risk factor for its occurrence. There has been long-standing interest in investigating this possible association. But, yet the evidence for such a relationship is far from conclusive. So, our study was aimed to investigate the relationship between *Toxoplasma* and *Toxocara* seropositivity in children with cryptogenic epilepsy.

2. Subjects and Methods

The present case-control seroprevalence study was carried out with a total of 132 consecutive pediatric age patients with cryptogenic epilepsy who were evaluated and followed up in the Neurology Unit at Mansoura University Children's Hospital, Mansoura, Egypt. This prospective study was conducted in the period from April 2012 to June 2013. Diagnosis of cryptogenic epilepsy was based on: a negative family history of epilepsy, no history of head trauma, previous meningitis/encephalitis or brain surgery and normal magnetic resonance imaging (MRI) study and electroencephalogram (EEG). All patients were subjected to full history taking and complete physical examination. The demographic and life style information was obtained through a survey questionnaire. The epileptic patients were classified into three age groups; group I (≤ 5 years), group II (> 5 years to ≤ 10 years), and group III (> 10 years to ≤ 14 years).

The control group consisted of 60 age matched volunteers with informed consent from parent/guardian from children attending out-patient clinic for other causes without any personal or family history of seizures. The study was approved by Ethics Committee of Pediatric department at Mansoura University Children's Hospital-Mansoura University.

2.1. Sample Collection

Three ml of venous blood was drawn aseptically from each subject included in the study. Serum was separated by centrifugation and each sample is divided into two tubes and stored at -20°C until tested.

2.2. Serological Investigation

Serum samples were tested for both anti-*Toxocara* IgG antibodies detection using RIDASCREEN *Toxocara*-IgG ELISA (R-Biopharm AG, Germany) kit which detects antibodies against the excretory/secretory antigen of the *Toxocara* larvae. For anti-*Toxoplasma* IgG antibodies detection, (Adaltis, Italy) kit was used. According to the manufacturer's recommendations, serum samples were diluted and used for testing; each serum sample was tested in triplicates. Positive and negative controls were included in the kit.

2.3. Statistical Analysis

Collected epidemiological, clinical and laboratory data were computerized and statistically analyzed using SPSS

16.0 (SPSS Inc., Chicago, IL.). Results were presented as frequencies [n (%)]. Chi-squared test was used in comparing the results. A p value of < 0.05 was considered significant.

3. Results

Of the 132 cryptogenic epilepsy patients included in the study, 62 (47%) were females and 70 (53%) were males; their age range was between 1-14 years (8.7 ± 3.9). The control group consisted of 60 subjects, 34 (56.7%) were males and 26 (43.3%) were females; their age range was between 1-14 years of age (9.9 ± 3.7). The epileptic patients and controls groups were quite similar regarding age groups and sex ($p > 0.05$). The overall seroprevalence of both *Toxoplasma* and *Toxocara* antibodies in both groups were 55.02 % and 47.91% respectively.

As regard *T. gondii* seroprevalence results, 80 epilepsy patients (60.6%) had statistically significant *T. gondii* seropositive rates, as compared to 26 (43.3%) seropositive controls ($P = 0.026$) (Table 1). Among *T. gondii* seropositive patients, there was male predominance (57.8%). Children residing in rural areas have higher *T. gondii* seropositivity than children residing urban areas (45 vs. 38).

Table 1. Seropositivity rates for both *T. gondii* and *Toxocara* spp

	Seropositivity	Patients (n) %	Controls (n) %	p value
<i>Toxoplasma gondii</i>	Positive	80 (60.6 %)	26 (43.3%)	0.026*
	Negative	52 (39.4%)	34(56.7%)	
<i>Toxocara</i>	Positive	64 (48.5%)	28 (46.7%)	0.815
	Negative	68(51.5%)	32(53.3%)	

*Statistically significant

As regard *Toxocara* results, 64 (48.5%) of children with cryptogenic epilepsy have positive anti-*Toxocara* antibodies in comparison to control ones 28 (46.7%). There is no significant association between epilepsy and *Toxocara* seropositivity ($p= 0.815$) (Table 1).

Table 2. Demographic characteristics of cases and control subjects

	Total Cryptogenic Epilepsy cases (n = 132)	Control (n = 60)	p Value
Sex			
Male	71(53.8%)	34 (56.7%)	0.710
Female	61 (46.2%)	26 (43.3%)	
Residency			
Urban	72(54.5%)	21(35%)	0.012*
Rural	60 (45.5%)	39(65%)	
Animal contact			
Yes	54 (40.9%)	19 (31.7%)	0.221
No	78 (59.1%)	41(68.3%)	

*Statistically significant

4. Discussion

Cryptogenic epilepsies are a group of epilepsy syndromes for which the etiology is hidden or occult [22]

and it comprises about 20% of all epilepsy syndromes [23]. Epidemiological links suggest an association between Helminth infection and epilepsy, particularly in the poorer areas of the world [24].

Toxoplasma gondii can form asymptomatic dormant cysts in the brain and could have the potential to cause epilepsy [7]. Also, the migrating *Toxocara* larvae can be found in the human brain causing an epileptic focus [25]. There is no precise report from both anti-*Toxocara* and anti-*Toxoplasma* antibodies in cryptogenic epileptic patients in our region. Our study screened the cryptogenic epileptic patients and the controls for *T. gondii* and *Toxocara* to investigate the possible correlation between these infections and cryptogenic epilepsy.

We found a statistically significant association between *T. gondii* infection and cryptogenic epilepsy was detected but no significant association between Toxocariasis and cryptogenic epilepsy. This significant correlation can be explained by either (i) dormant *T. gondii* cysts containing bradyzoites may cause epileptic foci and cryptogenic epilepsy in animal models it is proposed that some tissue cysts rupture and in doing so causes marked inflammation of that area and hence triggers microglial formation which may represent the 'tombstones' of *Toxoplasma* cysts and lead to scar tissue formation [26]; or (ii) The cryptogenic epilepsy patients could be more susceptible to this infections [27].

Regarding the association between *Toxoplasma* infection and cryptogenic epilepsy, our results are consistent with that of Yazar et al. [28] who have reported that the level of *T. gondii* IgG showed a statistically significantly high value in a study of cryptogenic epilepsy. And also with Stommel et al. [7] who suggested that chronic *T. gondii* infection with brain cysts may be a cause of cryptogenic epilepsy. On the other hand, Akyol et al. [29] reported no relationship between cryptogenic epilepsy and positive *T. gondii* serology in his study which was carried on 100 cryptogenic epileptic patients and 50 healthy volunteers that had no history of epilepsy in their first degree relatives.

However, we found no significant association between *Toxocara* infection and cryptogenic epilepsy. To our knowledge, there is no previous data about this relation except a study from Turkey which also did not find any association between cryptogenic epilepsy and seropositivity to *Toxocara* [29]. Also, there is less data about the association between *Toxocara* species and epilepsy. Most of them describe a significant association in the United States [29], Italy [21,30] and Iran [31]. In these studies, increased *Toxocara* seropositivity may be due to increased susceptibility to helminth infection.

Our findings demonstrated that the seropositivity to *Toxocara* and *Toxoplasma* cases were in the age group 5-10 years, the age where rate of infection is high and seropositivity were more in males than females as males are more frequently infected than females in most endemic areas [32]. As contact with cats and dogs is important risk factor of *Toxocara* infection [33,34]. We also asked about contact with animals in our cases but we found no significant relationship between animal contact and *Toxocara-Toxoplasma* seropositivity rates. We found that there was a significant relationship in antibody positivity against *Toxoplasma* and *Toxocara* and rural areas. This in accordance with that of [21,35,36] who have found that

the risk for developing *Toxocara* and *Toxoplasma* infection is higher in rural areas than in urban areas.

5. Conclusion

The study results have indicated that there was a statistically significant association between *Toxoplasma gondii* seropositivity and cryptogenic epilepsy and no significant association between *Toxocara spp* seropositivity and cryptogenic epilepsy. This correlation should be further evaluated. The results of the current study reemphasize the importance/significance of public health measures to prevent and control exposure of pediatric age/susceptible population to such parasitic infections.

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