

CLINICO-SEROEPIDEMIOLOGICAL EVALUATION OF TOXOCARIASIS IN EGYPTIAN EPILEPTIC CHILDREN

By

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Abstract

Toxocariasis is one of the commonest zoonosis parasites worldwide, particularly in sheep-raising countries. Epilepsy is an important health problem in developing countries. The study assessed the seropositivity of toxocariasis in Egyptian idiopathic epileptic children. A case-control study was conducted on 125 children (1-18 years) with idiopathic epilepsy (patients) and 72 non-epileptic normal children (controls). All children underwent history taking, clinical examination, lab investigations, ELISA, and WB seropositivity to detect toxocariasis.

The results showed no significant difference in age, sex, and socioeconomic level, but a significant difference was in residence and pet dogs between patients and controls ($p < 0.05$). Also, the *Toxocara* infection (ELISA) among patients was 41.6% versus 23.6% among controls and (Immunoblotting) 17.6% versus 5.5% respectively. There was an association between toxocariasis and epilepsy, with significant difference between patients and controls ($P < 0.05$). Seropositivity was more in epileptic with generalized than partial seizures ($p = 0.06$). As risk factors, residence and eating raw/undercooked meat showed significant difference and pet dogs and soil contact showed a highly significant difference in toxocariasis seropositive patients ($P < 0.05$).

Keywords: Egyptian children, Toxocariasis, Idiopathic epilepsy,

Introduction

Toxocariasis, a disease caused by larvae of *Toxocara canis*, *T. cati*, and/or congeners infection, represented the clinical syndromes in man including visceral and ocular larva migrans, neurotoxocariasis, and covert/ common toxocariasis (Rostami *et al*, 2019). In North Africa, since 1990, the seroprevalence studies and case reports were done in six countries: Algeria, Egypt, Libya, Morocco, Sudan, and Tunisia (Adeel, 2020). The toxocariasis risk factors were contact with dogs and cats, which hairs being a potential risk factor for zoonotic transmission (Rojas *et al*, 2017). Also, contaminated soil with dog feces, foods and water, poor hygiene, lower education levels, and poverty provided ideal transmission opportunities (Congdon and Lloyd, 2011). Infection occurs by ingestion of embryonated eggs in contaminated soil, water, or food where the eggs or larvae exist (Ma *et al*, 2018), raw or undercooked meat, or organs from paratenic hosts (cows, sheep, or chicken) (Yoshikawa *et al*, 2008). Ingested eggs gave juvenile larvae that cross the

small intestine and migrate to the somatic organs, preferably liver, brain, and eyes causing any of the four toxocariasis types: visceral, ocular, covert toxocariasis, and neurotoxocariasis with majority silent infections in human (Rubinsky-Elefant *et al*, 2010). The immunodiagnostic assays are enzyme-linked immunosorbent assay (ELISA) and Western blotting (WB), both using *Toxocara canis* excretory-secretory (TES) antigens (Schantz and Glickman, 1978).

Epilepsy is one of the commonest neurological disorders among children especially in developing countries (Fisher *et al*, 2014). Two types were known; idiopathic epilepsy with unknown etiology and secondary epilepsy which originates from cerebral lesions that may be traumatic, hypoxic, or infectious in origin (Fisher *et al*, 2005). According to the Commission on Classification and Terminology of the International League Against Epilepsy (ILAE, 1993), cryptogenic (Idiopathic) epilepsy was defined as epilepsy syndromes with an unknown etiology, but the suspected underlying brain disease was up to

60% (Manford *et al*, 1992).

Childhood epilepsy is one of the most important neurological disorders in both developing and developed countries. Epilepsy has an impact on both the mental and physical health of children. Epileptic children may suffer from behavioral or psychiatric disorders, or social isolation due to social stigmatization (Camfield and Camfield, 2015). Several studies have been carried out suggesting a possible role of toxocariasis in the incidence of epilepsy (Quattrocchi *et al*, 2012).

The current study aimed to assess the toxocariasis seropositivity in Egyptian epileptic children.

Subjects and Methods

Study design: A case-control study was performed on 125 children (1-18years) with idiopathic epilepsy (epileptic group) and 72 healthy children (control group) during the period from June 2019 to April 2020. The purpose and procedures were explained and written informed consent was obtained from all parents of the included children.

The epileptic cases were chosen from the Outpatient Clinic of Pediatric Neurology Unit, Department of Pediatrics, Al-Zahraa University Hospital, Cairo, Egypt.

Diagnosis was based on the definition (ILAE, 1993) depending on number of head injuries, number of CNS infections (meningitis or encephalitis) or brain surgery, and normal magnetic resonance imaging (MRI) study and electroencephalogram (EEG). Control children were cross matched on age and sex attending the Outpatient Clinic for other causes with negative personal or family history of seizures.

All cases and controls were subjected to history taking through a questionnaire including personal data and toxocariasis risk factors as contact with animals (dogs and cats), soil contact or eating soil (geophagia), and eating raw or undercooked meat or drinking unsanitary water. Complete general and neurological examinations were done for all participants.

Ethical consideration: The study was app-

proved by the Ethical Committee of Faculty of Medicine (Girls), Al-Azhar University.

Samples collection: Five milliliters of venous blood were taken from studied groups under complete aseptic conditions. Two ml of blood was collected in an EDTA tube to be used for the determination of differential leucocytic count for calculation of absolute eosinophilic count (Dacie and Lewis, 1992). The other 3 ml was centrifuged at 2000 rpm for 10 min., sera were kept at -20°C in sterile labeled aliquots for serological analysis.

Serological investigation: Sera were tested for anti-*Toxocara* IgG antibodies detection using RIDASCREEN *Toxocara*-IgG ELISA (R-Biopharm AG, Darmstadt, Germany) kit which detects antibodies against the excretory/secretory antigen of *Toxocara* larvae. Serum samples were diluted and used for testing; each serum sample was tested in triplicates. Positive and negative controls were included in the kit according to the manufacturer's instructions. WB technique used a commercial kit (*Toxocara* WB IgG, LDBIO Diagnostic, Lyon, France). Strips were incubated with 1/100 diluted sera for 2hr at room temperature than washed three times in PBS containing 0.1% Tween 20, and then re-incubated for 2hr with second antibody, anti-human IgG peroxidase conjugate (1:1,000). Strips were washed again, diaminobenzidine was added and reaction was stopped by several washes in distilled water. Reaction was considered positive when it reacted with 2 or more of the supplied low-molecular-weight bands (LMWB; 24-35 kDa).

Statistical analysis: Data were coded, tabulated, and computerized for analysis using SPSS 18.0 (SPSS Inc., Chicago, IL.). Results were presented as frequencies and percentages. A chi-squared test compared data, and significant was at P-value < 0.05.

Results

The 125 idiopathic epilepsy children were 66 (52.8%) males and 59 (47.2%) females with ages ranged between 1-18 years (7.6±2.3). The control was 72 healthy children, 39 (54.2%) were males and 33(45.8%)

females with cross-matched ages, sex, and socioeconomic levels, but with a significant difference ($p < 0.05$) in residence and dog ownership (Tab. 1).

Table 1: Sociodemographic data of groups

Factors		Epileptic children (n=125)	Control (n=72)	P-value
Age (Mean \pm SD)		7.6 \pm 2.3	7.2 \pm 3.1	0.3
Sex	Male	66(52.8)	39(54.2)	0.85
	Female	59(47.2)	33(45.8)	0.88
Socioeconomic level	Moderate	58(46.4)	43(59.7)	0.72
	Low	67(53.6)	29(40.3)	
Residence	Urban	45(44%)	41(56.9)	0.003**
	Rural	80(56%)	31(43.1)	
Dogs /puppies ownership		78(62.4%)	19(26.4%)	0.0001**

Epileptic children (29.6%) partial seizures and (70.4%) generalized seizures, On age was (4%) below 2 years, (62.6%) between 2-5 years and (33.4%) above 5 years. Others (12%) respiratory, (5.6%) hepatic and gastrointestinal, and (1.6%) ocular (Tab. 2).

Table 2: Clinical findings in epileptic group

Epileptic children		Frequency (%)
Epilepsy type	Partial seizures	37 (29.6%)
	Generalized seizures	88 (70.4%)
Epilepsy age of onset	<2 years	5 (4%)
	2 – 5 years	78 (62.4%)
	>5 years	42 (33.6%)
Respiratory symptoms (s&sx) Fever, dyspnea, cough, chest pain, and wheezing		15 (12%)
Hepatic & GIT symptoms (s&sx) Abdominal pain, anorexia, nausea, vomiting, & hepatomegaly		7 (5.6%)
Ocular symptoms (s&sx) Diminished vision/visual acuity, red-eye, and leukocoria		2 (1.6%)

Epileptic children showed 52 (41.6%) *Toxocara* ELISA-positive compared to controls 17 (23.6%), with a significant difference ($P = 0.011$). Immunoblotting *Toxocara*-IgG positive were 22(17.6%) among epileptic children compared to controls 4 (5.5%), with significant difference ($P = 0.016$). Epileptic children showed 60 (48%) eosinophilia compared to controls 17 (23.6%) with a highly significant ($P < 0.05$) difference (Tab. 3).

Table 3: laboratory findings (eosinophilia and serology) in groups

Laboratory Investigations		Epileptic children (n=125)	Control (n=72)	P-value
<i>Toxocara</i> -IgG ELISA	seropositive	52 (41.6%)	17(23.6%)	0.011*
	seronegative	73 (58.4%)	55(76.4%)	
<i>Toxocara</i> -IgG Immunoblotting	seropositive	22 (17.6%)	4(5.5%)	0.016*
	seronegative	103 (82.4%)	68(94.4%)	
High Eosinophilia		60 (48%)	17(23.6%)	0.0007**

Residence and eating raw/uncooked meat and soil showed a highly significant difference as toxocariasis risk factor (Tab. 4).

Table 4: Risk factors and epilepsy type in *Toxocara* seropositive epileptic group

Risk Factors		IgG positive (n=22)	IgG negative (n=103)	P-value
Age	<6yrs	13(59.1%)	58(56.3%)	0.81
	\geq 6yrs	9(40.9%)	45(43.7%)	
Sex	Male	12(54.5%)	53(51.5%)	0.79
	Female	10(45.5%)	50(48.5%)	
Socioeconomic level	Moderate	7(31.8%)	53(51.4%)	0.09
	Low	15(68.1%)	50(48.5%)	
Residence	Urban	8(36.4%)	24(55.3%)	0.01*
	Rural	14(63.6%)	56(44.7%)	
Soil contact/geophagia (pica)		20(90.9%)	24(23.3%)	0.0001**
Dog Contact		19(86.4%)	56(54.4%)	0.0001**
Eating raw or undercooked meat		7(31.8%)	12(11.7%)	0.016*
Epilepsy type	Partial seizures	7(31.8%)	30(29.1%)	0.65
	Generalized seizures	15(68.2%)	73(70.9%)	

Discussion

Epilepsy is an important health problem, up to 80% of patients live in low or middle-

income countries (WHO, 2019), with correlated between helminthes and epilepsy especially in developing countries (Wanger and

Newton, 2009).

In Egypt, the toxocariasis was encountered in man, edible animals and stray or pet-dogs (Khalil, 1977; Haridy *et al.*, 2009; Morsy, 2020). Visceral toxocariasis occurred primarily in young children, especially between 2-7 years old (Stewart *et al.*, 2005). Three most common organs or systems affected by *Toxocara* are the lungs, liver, and central nervous system. Presenting symptoms may be not specific as fever, fatigue, anorexia, and lymphadenopathy. Pulmonary symptoms were (cough, dyspnea, or wheezing) and abdominal symptoms (pain, hepatomegaly, or splenomegaly) when larvae migrate to lungs or abdominal organs, respectively, or CNS and eye causing ocular and neurotoxocariasis (Marx *et al.*, 2007).

In the present study, there was no significant difference in age, sex, and socioeconomic level between epileptic children and controls. This agreed with Khademvatan *et al.* (2014), and Salama *et al.* (2019) who did not find difference as to age or sexes or economics among *Toxocara* positive epileptic patients, without differences in urban or rural areas. But, Eraky *et al.* (2016) reported more epileptic cases in rural areas.

In the present study, epileptic children showed (29.6%) partial seizures compared to (70.4%) generalized seizures, with (4%) onset < 2years, (62.6%) between 2-5years and (33.4%) > 5 years, with respiratory (12%), hepatic and gastrointestinal (5.6%), and ocular (1.6%) symptoms. This agreed with Salama *et al.* (2019) found that the seizures type was more among toxocariasis epileptic children compared to generalized ones but, without significant difference between them. Akyol *et al.* (2007) found non-significant difference between focal and generalized epilepsy, but, Nicoletti *et al.* (2008) found a significant association between toxocariasis and epilepsy, especially in partial epilepsy.

In the present study, ELISA-*Toxocara* positives were 41.6% in epileptic children versus 23.6 in controls and 17.6% versus 5.5% of control, and WB positively correlated bet-

ween toxocariasis, and epilepsy ($P < 0.05$). The agreed with Temsah *et al.* (2021) who found association between *T. canis* and bronchial asthma, chronic urticaria, and either unexplained cause of epilepsy or focal neurologic deficits.

Nicoletti *et al.* (2002; 2007; 2008) in Bolivia, Burundi, and Italy respectively, found significant association between anti-*T. canis* antibodies among epileptic patients and controls. Also, Kamuyu *et al.* (2014) in Sub-Sahara of Africa correlated between pathogenesis of epilepsy/toxocariasis patients. *Toxocara* larvae stimulated granuloma formation around them that caused acute symptomatic seizures and then left after resolution of fibrous scars and chronic granulomatous lesions leading epilepsy (Wagner and Newton, 2009). The epilepsy autoimmune nature was due to antibodies to CNS major excitatory neurotransmitter (Levite and Ganor, 2008) stimulated by toxocariasis autoantibodies (Obwaller *et al.*, 2004). Again, Winkler *et al.* (2008); Zibaei *et al.* (2013); Allahdin *et al.* (2015); El-Tantawy *et al.* (2013); Eraky *et al.* (2016) and Salama *et al.* (2019) neither found association between *Toxocara* positivity nor cryptogenic epileptic children or controls.

In the present study, high eosinophilia was in epileptic children (48%) and in controls (23.6%) with a high significant difference ($P = 0.0008$). This agreed with Kwon *et al.* (2006), and Karadam *et al.* (2008) who reported that toxocariasis elevated eosinophilia in both peripheral blood and internal organs, with the marked elevation of IgM (Elsheikha *et al.*, 2008).

In the present study, significant difference was among eating raw/undercooked meat, dogs, and soil contact as risk factors for toxocariasis/epilepsy ($P < 0.05$). This agreed with Arpino *et al.* (1990) and Despommier *et al.* (2003) who reported that geophagia or soil eating pica increased toxocariasis risk. Also, Arpino *et al.* (1990); Fernando *et al.* (2007) and Salama *et al.*, 2019) correlated between pet-dog and toxocariasis. But, Aky-

ol *et al.* (2007) and El-Tantawy *et al.* (2013) did not find relationship between pica and toxocariasis in cryptogenic epileptic patients. Also, Fan *et al.* (2015) correlated between eating raw meat or liver and toxocariasis. But, Salama *et al.* (2019) did not find significant difference among positive or negative epileptic patients and raw meat.

Conclusion

Toxocariasis is worldwide zoonotic disease. The outcome data proved significant correlation between toxocariasis and epilepsy. Dogs and soil contact were major risk factors in rural areas. Health education and preventive measures were recommended.

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