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RESEARCH

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Seroprevalence of toxoplasmosis among children with autism

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Abstract

Background Toxoplasmosis is considered one of the most common public health troubles. Among these health troubles, toxoplasmosis was recently linked to many neuropsychiatric and behavioral disorders, especially autism spectrum disorder (ASD). The purpose of this study was to determine the seroprevalence of toxoplasmosis in children with ASD.

Methods The study was conducted on 100 children, grouped in two groups; group 1 (patient group) included 50 children with ASD and group 2 (control group) included 50 healthy children. ASD in the patient group was diagnosed according to DSM 5 criteria of ASD. Every child involved in this study underwent a history taking, a clinical examination, and laboratory investigations to detect serum anti-Toxoplasma IgG and IgM antibodies using ELISA. Children of the patient group were further assessed using the Childhood Autism Rating Scale to evaluate the severity of their symptoms.

Results The seroprevalence of IgG among ASD children was highly significant compared to the healthy children. The detected difference between the 2 groups regarding seroprevalence of anti-Toxoplasma IgM antibodies was insignificant. No significant correlation could be demonstrated between Toxoplasma infection and severity of autistic symptoms in the ASD group. Furthermore, the study revealed an increase in anti-Toxoplasma IgG antibodies in ASD children with positive family history of ASD rather than those with no such history. In addition, an increase in seroprevalence of both anti-Toxoplasma antibodies among children with low socioeconomic standards compared to children with moderate or high standards.

Conclusions The study revealed that the old but not the recent infection with Toxoplasma in children could be linked to their ASD.

Keywords Autism spectrum disorder, Toxoplasma gondii, ELISA, Seroprevalence

Background

Autism spectrum disorder (ASD) is a group of complex neurodevelopmental disorders. Early onset difficulties in social interaction, repetitive behavior, and verbal and non-verbal communication are characteristics of ASD. Numerous elements, such as fetal testosterone levels, immunological imbalance, environmental variables, obstetric problems, intrauterine infections, and genetic background, have been linked to the etiology of ASD [1]. Males are more likely than females to suffer from autism [2].

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There is proof linking some prenatal infections in mothers, such as toxoplasmosis, to neurodevelopmental abnormalities including ASD in children. Nevertheless, it is still unknown whether toxoplasma gondii and autism are related [3].

Toxoplasmosis is considered to be an epidemic disease spreading widely all over the world and became a global health hazard, as infection in human is reported among 30 to 50% of human population in the world [4].

Toxoplasma gondii affects the hypothalamic–pituitary–adrenal processes, interacts with particular genes, and affects the production of neurotransmitters, it also has a great affinity for brain tissue and may result in neuronal injury [5].

The effect of infection with a Toxoplasma gondii in the early period of life happens at the neurodevelopmental stage. Thus, children are specifically at a high risk [6]. Because Toxoplasma primarily affects the central nervous system (CNS), infection in childhood may result in neurodegeneration, impairing CNS function. This theory is supported by the finding that children and adolescents who struggle in school have a greater rate of Toxoplasma antibodies than children and adolescents who are developing normally at the same age [7].

One of the most used indirect diagnostic techniques is the enzyme-linked immunosorbent test (ELISA) [8]. Because of its great sensitivity, affordability, and ease of use, ELISA is frequently used to diagnose Toxoplasma gondii Furthermore, by identifying specific anti-Toxoplasma gondii Immunoglobulin M (IgM) and Immunoglobulin G (IgG), respectively, it may identify the recent active infection as well as the old one [9].

As a trial to shed light on the potential association between Toxoplasma gondii infection in children and ASD, the following work plan was considered for detection of anti-Toxoplasma gondii antibodies prevalence in their blood compared to healthy children.

Methods

To demonstrate any association between ASD and toxoplasma infection in children, a hospital-based case–control study was conducted in the Child Psychiatry Unit at Badr Hospital and the Clinical Pathology Department, Faculty of Medicine, Helwan University. The study extended over six months duration from July 2020 until January 2021.

Subjects

Taking into account the 3% prevalence of positive IgG for Toxoplasma gondii in the general population and $\alpha = 0.05$ at a 95% confidence interval (CI), we utilized this formula $[n = Z2\ P\ (1-P)\ /d2]$ to determine the appropriate sample size. Where n is the sample size, Z is the statistic corresponding to the degree of confidence, P is the predicted prevalence, and d is precision. We calculated that we would require 50 ASD children with a power of 80% [10].

The study included two groups: patient group and control group. The patient group consisted of 50 children with ASD aged 3–12 years, they were recruited from the child psychiatry clinic attendants at Badr University Hospitals. Children with comorbid medical or psychiatric disorders were excluded.

Another 50 healthy age and sex-matched children were included in the study. They were selected from siblings of children in the patient group. Children with medical or psychiatric disorders were excluded.

In addition, children from both groups whose parents did not give their consents were also excluded from the study.

Methods

The Helwan University Faculty of Medicine Ethics Committee evaluated and authorized this analytical study (License no. 29-2020). After explaining the process and the purpose of the study, the parents of the children were asked for their written agreement. All participants were given explanations about the nature of the study, and the process did not provide unnecessary pain or discomfort. The results of the investigations were given to the patient's legal guardian. The leftover specimens were discarded according to the biosafety instructions.

To gather data, a pre-made questionnaire was given to each child involved in the study, and they were also put through the following procedures:

1. History taking including personal information, family history, past and present history of any general, neurological, and/or mental diseases.
2. Clinical examination and neurological evaluation.
3. Psychiatric examination by an expert psychiatrist to confirm the ASD diagnosis and exclude any mental comorbidities in the patient group and to exclude any psychiatric morbidity in the control group. ASD diagnosis of the patient group was according to Diagnostic and Statistical Manual of Mental health, 5th Edition (DSM 5) criteria of ASD [11].

The children of the patient group were further assessed using The Childhood Autism Rating Scale (CARS) to evaluate the severity of autism [12]. CARS is a 15-item scale used to identify children (over age 2 years) with autism and to distinguish mild to moderate from severe autism. Severe autism is diagnosed when the score is 37 or more. Items of the CARS are; relation to people, imitation, emotional response, body use, object use, adapting

to change, visual response, listening response, taste—smell and touch—tears or nervousness, verbal communication, non-verbal communication, activity level, level of intellectual response and finally general impression. The version of CARS administered in this study was the Arabic version that was translated by El Dafrawi [13] and has been widely used in the Arab countries for clinical and research purposes.

Blood samples collection and processing

A sterile disposable hypodermal syringe with a 23-gauge needle was used to draw 5 ml of venous blood from each study participant. The syringe barrel was then filled with sterile tube with the patient's name and the collection date. Centrifugation at 1000 rpm was used to separate the serum from the whole blood, which was then frozen at -80 °C until serological analysis [14].

Laboratory investigations (serological test)

Using an enzyme-linked immunosorbent assay with a commercially available kit (EDITM Toxoplasma gondii IgM and IgG ELISA kit, Epitope Diagnostics, Inc., San Diego, USA), anti-Toxoplasma gondii IgM and IgG were detected in sera samples in accordance with the manufacturer's instructions. Interpretation and reading of the result of ELISA was done using both the visual and ELISA spectrophotometric microplate reader (Tecan: Sunrise:16039400) capable of reading absorbance at 450 nm**.** The lack of toxoplasma gondii IgG and IgM antibodies suggests that there has never been an infection, either recently or in the past. Evidence of a previous Toxoplasma gondii infection is indicated by the presence of Toxoplasma gondii IgG antibodies in the absence of IgM antibodies. When Toxoplasma gondii IgM antibodies are found without IgG antibodies, this indicates a recent Toxoplasma gondii infection [15].

Statistical analysis

IBM SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA) and NCSS 11 for Windows (NCSS LCC., Kaysville, UT, USA) were used to analyze the data. Mean ± standard deviation (SD) was used to express the quantitative data, while frequency and percentage were used to express the qualitative data. When comparing two means of non-normally distributed data, the Mann Whitney *U* test was employed [16]. The proportions between two qualitative measures were compared using the Chi-square (X^2) test of significance [17]. Probability (p value): p values less than 0.05 were regarded as significant, p values less than 0.001 as extremely significant, and p values greater than 0.05 as inconsequential.

Results

Sociodemographic data of the participants

It was demonstrated that there was no significant difference between ASD and control groups regarding age, gender, residence and socioeconomic status. (Table 1).

Seroprevalence of Toxoplasma infection and optical densities of seropositive anti-*Toxoplasma* antibodies among ASD and control groups

A highly significant difference was detected in seroprevalence of IgG between autistic children and controls (16/50) (4/50), respectively (p=0.005) while optical densities were not significantly different between the IgG positive ASD children and healthy children (p>0.05) (Table 2). There was no significant difference in seroprevalence of IgM between ASD and control groups (2/50) (1/50), respectively (p=0.503) as well as the optical densities which were also not significantly different between the IgM positive autistic children and healthy children (p>0.05). (Table 3)

Relation between the infection of Toxoplasma gondii among ASD children and severity of their symptoms

Regarding severity of autism, 22 children out of 50 ASD children demonstrated a mild to moderate degree of autism according to CARS classification and 28 children demonstrated severe degree of autism, there was insignificant increase in IgG positivity prevalence between ASD children with mild to moderate autism (9/22) than children with severe autism (7/28) (p = 0.48) (Table 4).

Table 1 Sociodemographics of both patient and control group

	ASD group (no. = 50)	Control group (no. = 50)	u/χ^2	p value
Age				
Mean ± SD	5.96 ± 2.23	5.5 ± 1.7		
Median	5	5		
Range	3–12	3–11	0.752	0.452
Gender				
Male	33 (66%)	24 (48%)	3.3	0.069
Female	16 (34%)	26 (52%)		
Residence				
Urban	6 (12%)	10 (20%)	1.19	0.27
Rural	44 (88%)	40 (80%)		
Socioeconomic				
High	1 (2%)	4 (8%)		
Moderate	13 (26%)	11(22%)	1.98	0.371
Low	36 (72%)	35 (70%)		

u = Mann–Whitney U test of significance

χ^2= Chi-square test

p > 0.05 = insignificant

Table 2 Seroprevalence of anti-Toxoplasma gondii IgG antibodies and optical densities of seropositive IgG anti-Toxoplasma gondii antibodies among patient and control groups

	ASD group (no. = 50)		Control group (no. = 50)		u/χ^2	*p* value
	No.	%	No.	%		
Seroprevalence of Anti-Toxoplasma gondii IgG infection					10.78	0.005
Positive	16	32	4	8		
Equivocal	3	6	1	2		
Negative	31	62	45	90		
Optical density of seropositive IgG cases					0.32	0.75
Mean ± SD	0.1343 ± 0.016		0.1375 ± 0.026			
Median	0.132		0.136			
Range	0.11—0.164		0.113—0.166			

u = Mann–Whitney *U* test of significance

χ^2= Chi-square test

p > 0.05 = insignificant

Table 3 Seroprevalence of anti-Toxoplasma gondii IgM antibodies and optical densities of seropositive IgM anti-Toxoplasma gondii antibodies among ASD and control groups

	ASD group (no = 50)		Control group (no = 50)		*u*/χ^2	*p* value
	No.	%	No.	%		
Seroprevalence of Anti-Toxoplasma gondii IgM infection					1.375	0.503
Positive	2	4	1	2		
Equivocal	1	2	0	0		
Negative	47	94	49	98		
Optical density of seropositive IgM cases						
Mean ± SD	0.138 ± 0.006				1.225	0.22
Median	0.138		0.115			
Range	0.134–0.143					

u = Mann–Whitney *U* test of significance

χ^2= Chi-square test

p > 0.05 = insignificant

In addition, no significant difference was detected in the prevalence of IgM positivity as the only 2 IgM positive cases demonstrated a severe degree of autism (Table 5).

Relation between the prevalence of the Toxoplasma gondii infection among ASD children and their family history of autism

Concerning family history of autism, 22 children of ASD children have a positive family history of autism, while 28 of ASD children have no family history of autism. There was a significant increase ($p = 0.001$) in IgG positivity prevalence between ASD children with positive family history (13/22) than children with no family history (3/28), while it was statistically insignificant in the prevalence of IgM positivity between the same groups (1/22) (1/28), respectively ($p = 0.51$).

Relation between the prevalence of Toxoplasma gondii infection among ASD children and their socioeconomic status

According to socioeconomic status only one child of the autistic children demonstrated high social class while 13 children were moderate class and 36 were low class and the study revealed that ASD children from lower socioeconomic classes had higher prevalence of both old (IgG positivity) and recent (IgM positivity) toxoplasmosis than

Table 4 Relation between the prevalence of anti-Toxoplasma gondii IgG positive ASD children and their severity of symptoms according CARS score

		Severity of autism		χ2	*p* value
		Mild to moderate (no = 22)	Severe (no = 28)		
Anti-Toxoplasma gondii IgG					
Positive	No.	9	7	1.46	0.48
	%	40.9	25		
Equivocal	No.	1	2		
	%	4.5	7.1		
Negative	No.	12	19		
	%	54.5	67.9		

χ^2= Chi-square test

$p > 0.05$ = insignificant

Table 5 Relation between the prevalence of anti-Toxoplasma gondii IgG positive ASD children and their severity of symptoms according CARS score

		Severity of autism		χ2	*p* value
		Mild to moderate (no. = 22)	Severe (no. = 28)		
Anti-Toxoplasma gondii IgM					
Positive	No.	0	2	2.4	0.33
	%	0	7.1		
Equivocal	No.	1	0		
	%	4.5	0		
Negative	No.	21	26		
	%	95.5	92.9		

χ^2= Chi-square test

$p > 0.05$ = insignificant

did children from higher or moderate socioeconomic classes, yet this increase was statistically insignificant.

Discussion

Toxoplasmosis is a cosmopolitan parasitic disease that infects humans and other warm-blooded animals [18]. According to studies, immunocompromised individuals and pregnant women have a high prevalence of toxoplasmosis infections [19]. Pregnancy-related infections, particularly in the early stages, have been linked to neurodevelopmental problems, mainly ASD [20].

The purpose of the current study was to ascertain the seroprevalence of T. gondii infection in children with ASD to report a connection between toxoplasmosis and autism in children. Serum anti-Toxoplasma gondii IgM and IgG levels in ASD children were measured using ELISA and compared to healthy children in the same age group.

The current work revealed that the total cases of toxoplasmosis among ASD children examined were 18/50 (36%) cases, compared to only 5/50 (10%) cases detected in non-autistic children, and this difference between the two groups was highly significant ($p = 0.004$). Sixteen (32%) out of the 50 autistic children (12 males and 4 females) demonstrated an old infection (IgG + ve) with Toxoplasma gondii, while only 4/50 children (8%) among the control healthy children were found positive for an old T. gondii infection, and the difference was highly significant between the 2 groups. Only 2 (4%) out of 50 autistic children were found to have a recent infection (IgM + ve) with Toxoplasma gondii, and this was compared to only one child (2%) found positive for anti-T. gondii IgM antibodies among the 50 control healthy children.

The current study's findings were consistent with a study by Prandota et al. [19], which showed that 11 (23.9%) out of 46 autistic children were positive for serum anti-Toxoplasma gondii IgG antibodies, whereas only 2 (4%) children were positive among a matching control group.

To determine the seroprevalence of toxoplasmosis, Al Malki et al. [20] tested 36 blood samples from mothers, 36 from their non-autistic children, and 36 from their autistic children. Using an ELISA technique, they found that 33.34% of people with autism had toxoplasmosis (5.56% IgG + /IgM + , 11.11% IgG−/IgM + , and 16.67% IgG + /IgM−).

According to the findings of a meta-analysis conducted by Nayeri et al. [21], toxoplasmosis was linked to an increased risk of autism, with a higher seroprevalence of anti-Toxoplasma gondii IgG antibodies in autistic patients compared to control groups. Toxoplasma gondii was also thought to be a risk factor for the development of autism.

In addition, Flegr and Horáček [22] found a higher seroprevalence rate of toxoplasmosis in autistic patients than among healthy individuals. The study conducted by Hamid et al. [23] evaluated the seroprevalence of toxoplasmosis in children with autism and normal children. Fifty autistic children and fifty normal children, aged three to twelve, were included in the sample. The findings demonstrated that, in comparison to the normal group, children with autism had higher rates of toxoplasmosis.

Similar results but with a low seroprevalence rate were reported by Esnafoglu et al. [24] who found only 3 (2.9%) autistic children were positive for anti-Toxoplasma gondii

IgG and 1 (2%) control were positive with no significant difference.

In 2017, Spann et al. [3] showed that women had high levels of Toxoplasma gondii IgM antibody, and their autistic children had high levels of IgG avidity. The results indicated a connection between childhood autism and maternal Toxoplasma gondii antibodies.

However, in a study done by Afsharpaiman et al. [25] to assess the positive serology of Toxoplasma gondii in autistic children, all tested autistic children were negative for anti-Toxoplasma gondii IgG and IgM.

The contradictory results of some studies reporting many varied seroprevalence rates compared to our study could be explained by the different environmental conditions, different sample sizes, and even different races of the candidates used in every study. Furthermore, changes in seroprevalence may result from variations in mothers' attributes, such as how they manage their cats, their educational background, their hygiene practices, and their feeding habits, as well as variations in climatic conditions (rainfall, temperature, soil type, altitude, and dry climate) [26].

As regards the correlation between the severity of autism (as demonstrated by CARS) and the seroprevalence of toxoplasmosis among the study group, our study revealed that children with autism who were classified as mild to moderately CARS were more likely to have old toxoplasmosis than those classified as severely CARS, but this difference was insignificant. In addition, there were insignificant differences between the recent infection of Toxoplasma gondii among autistic children and their CARS classification. Similarly, Prandota et al. [19] found no discernible variation in the severity of autism in children with and without toxoplasmosis or in the seropositivity of toxoplasmosis. However, Esnafoglu et al. [24] did discover a noteworthy association between the CARS degrees of the case and control groups.

The current study's findings on a family history of autism showed that in comparison to children without a family history of autism, children with autism who had a positive family history had a significantly higher prevalence of old toxoplasmosis. However, such a significant difference was not detected among autistic children with recent toxoplasmosis. This comes in agreement to Baioumy et al. [27] who reported a significant difference between the seroprevalence of old and recent toxoplasmosis and a positive family history of another psychiatric disorder which is schizophrenia among their study population.

It appears that the strong correlation between anti-Toxoplasma gondii IgG antibodies and the etiology of several neuropsychiatric illnesses in general and autism in young children in particular play an important role with the need for more attention to the prenatal and postnatal screening of both mothers and their offspring. This could shed insight on the role of latent toxoplasmosis in the etiology of many neuropsychiatric disorders in mothers and their offspring.

It's possible that toxoplasmosis contributes significantly to the etiopathogenesis of mental health illnesses, as proposed by many authors who suggested varied mechanisms through which Toxoplasma gondii parasites could affect the brain, such as Horáček et al. [28] who stated that the effect of Toxoplasma gondii on developing psychiatric disorders was most probably due to the immune response of the brain and the release of mediators like interferon-gamma. In addition, it was thought that Toxoplasma gondii increases dopamine and parasitic tyrosine hydroxylase, which increases anxiety expression [29].

An experiment done on immunocompromised and experimentally infected rodents revealed marked changes in the physiology of the rodent's brains with seizures attacks. These changes in CNS physiology could result from direct parasite interactions, such as effector protein injection or persistence within a host cell, indirect consequences of the immune system's attempt to contain the infection, or a combination of the two. Though seizures are known to occur in both congenitally infected individuals and acquired immunodeficiency syndrome (AIDS) patients with toxoplasmic encephalitis, it is still unknown how these findings transfer to human consequences [30].

Undoubtedly, significant progress in examining the potential roles of Toxoplasma gondii in the emergence of mental illnesses will be required. This will aid in the prevention and enhancement of the treatment of mental and behavioral illnesses as well as the development of preventative strategies to lessen the pathogenic mechanisms linked to Toxoplasma gondii infection.

Large-scale research is also necessary to determine the etiopathological relationship between Toxoplasma gondii infection and mental problems, as well as the possible mechanisms influencing parasite alterations in learning and memory processes in humans and rodents.

Conclusions

From the data obtained in the present study, we concluded that there is an association between old, but not recent toxoplasmosis and ASD in children, yet there is no correlation between toxoplasmosis and severity of ASD symptoms in these children. In addition, this study demonstrated that ELISA was a good screening test and useful for sero-epidemiological surveys of toxoplasmosis, as it is commercially available, safe, simple, an easy technique, and not time-consuming.

Abbreviations

AIDS	Acquired immunodeficiency syndrome
ASD	Autism spectrum disorder
CARS	Childhood Autism Rating Scale
CI	Confidence interval
CNS	Central nervous system
DSM 5	Diagnostic and Statistical Manual of Mental health, 5th Edition
ELISA	Enzyme-linked immunosorbent assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
SD	Standard deviation

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Author contributions
SE. proposed the research idea and design, supervised the practical part of the study, and revised the manuscript. KA. participated in the concept and design of the study and supervised the whole research process. EA. collected the data, performed the data analysis, and contributed to the interpretation and editing of the manuscript. HH. diagnosed the cases and applied the CARS and helped in editing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The Helwan University Faculty of Medicine Ethics Committee evaluated and authorized this analytical study (License no. 29-2020). After explaining the process and the purpose of the study, the parents of the children were asked for their written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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