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ORIGINAL ARTICLE

Detection of Anti *Toxoplasma* antibodies in children with autism in Shebin Al-Kom district Menoufia Governorate, Egypt

Marwa A. Gouda*, Dalia Shafey
Clinical and Molecular Parasitology Department, National Liver Institute (NLI), Menoufia University, Shebin Al Koom, Menoufia, Egypt

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****Corresponding Author:***
Marwa Ahmed Gouda Ali Youssef
Clinical and Molecular Parasitology Department, National Liver Institute (NLI), Menoufia University, Shebin Al Koom, Menoufia, Egypt
Tel.: 01003080991
Marwa_goda@liver.menofia.edu.eg

ABSTRACT

Background; *Autism spectrum disorders (ASD), are a range of mental disorders of neurodevelopmental type.* More than three billion human hosts are estimated to be affected by the protozoan parasite *Toxoplasma gondii*, which is intracellular pathogen with a particular preference for the central nervous system (CNS). *Accumulating evidence suggests that latent chronic toxoplasmosis* contributes to *triggering and progression of many neurological and psychiatric disorders, however the link and prevalence in ASD is not clear.* ***Objective;*** *The present study aimed to detect the relationship between Toxoplasma gondii infection in children suffering from autism disorder.* ***Method:*** *A case-controlled study was conducted on 80 children (40 cases and 40 controls), aged from 4-10 years. They were tested for the presence of anti-T. gondii. antibodies by ELISA. Analysis of data was performed using the Chi-square test and Fisher's exact test.* ***Results****; No significant differences were detected between healthy (5.0%) and diseased children regarding presence of anti-toxoplasmosis antibodies (17.5%). However a significant difference was observed between boys and girls concerning age and seroprevalence of toxoplasmosis, the percent of autistic boys with toxoplasmosis was 9.1%, while the infection was represented in 57.1% of girls with autism. History of acquiring infection with toxoplasmosis during pregnancy was positive in 27.5% of diseased children compared to control children where the percent was 12.5%, with no statistically significant result. Considering residence, there were no significant differences in the seroprevalence of T. gondii IgG among urban and rural regions (P-value =1.0).* ***Conclusion:*** *From our study, we concluded that despite the absence of significant difference between healthy and diseased children in regard to toxoplasmosis, autistic children were highly risky for catching toxoplasmosis infection which need more research in this era.*

INTRODUCTION

Toxoplasmosis is one of the most common parasitic infections in humans and other warm-blooded vertebrates including marine mammals, birds, and livestock. It has infected approximately one-third of the world's human population[1]. The most common form of infection is latent toxoplasmosis (asymptomatic)[2], it can induce different hormonal and behavioral alterations in humans and rodents[3]. It is also, recognized as being involved in the etiology of various psychotic disorders[2,4-6].

Humans usually get infected by consumption of undercooked meat, unwashed or poorly washed vegetables, or contaminated drinking water[7]. After a short phase of acute toxoplasmosis, the infection becomes latent and becomes encysted in the central nervous system and muscle tissues, potentially through the whole life of the infected host[8,9]. The parasite can alter the behavior of the host to increase the chance of transmission[10]. Some studies have found that changes in infected individuals' personality characteristics were referred to toxoplasmosis infection[11,12].

Autism spectrum, also known as Autism Spectrum disorder (ASD), is a range of mental disorders of the neurodevelopmental type. It includes autism and Asperger syndrome. Autism is a developmental disorder characterized by difficulties in social interaction, communication, and the presence of restricted and repetitive behavior. Parents usually notice signs concerning developmental milestones during the first three years of their children live[13,14].

The cause of ASD is uncertain. Risk factors include having an older parent, a family history of autism, and certain genetic conditions. Infections and family history was estimated between 64% and 91% of risk agents. The diagnosis is based on symptoms. The DSM-5 redefined the autism spectrum disorders to encompass the previous diagnoses of autism, Asperger syndrome, pervasive developmental disorder not otherwise specified (PDD-NOS), and childhood disintegrative disorder[15,16].

The association between toxoplasmosis and autism spectrum disorder was under research lately.

The present study aimed to detect the seropositivity of *Toxoplasma* infections in patients with autism, in Shebin Al Kom, Menoufia, Governorate, Egypt.

METHODOLOGY

Study design:

Our study was designed as a case-control, eighty children were included, 40 of them had autism and another matched 40 children for age and gender, served as control. The age of participants ranged from four to ten years. They were chosen from different speech centers in Shebin Al Kom district, Menoufia Governorate, Egypt. The control was randomly chosen. Neither of the two groups was immunodeficient nor presented by any other major psychiatric disorders or neurological diseases. Diagnosis of autism was established by the childhood Autism Rating Scale (CARS) (17). The study was performed over 12 months from February 2016 to February 2017.

Ethical consideration:

The research was approved by the Research Ethics Committee, Faculty of Medicine, Menoufia University. Parents of cases and control groups were asked to agree on performing the study in a written consent after describing the procedures, and purpose of the study.

Sample collection:

A blood sample of 5 mL was drawn from each child to test for the presence of toxoplasmosis infection. Samples were left to clot first then, they were separated by centrifugation at 3000 rpm for 15 min and reserved at −20°C refrigerator for testing toxoplasmosis infection.

Serological test:

Measurement of the serum level of Anti-*Toxoplasma* antibodies (IgG and IgM) was done using enzyme-linked immunosorbent assay (ELISA) technique according to manufacturer protocol (IgG and IgM: Trinity Biotech Captia, USA).

Questionnaire sheet:

A complete questionnaire was taken from the guardians of both the control group and cases, to obtain demographic data info in details about age, gender, socioeconomic status, and residence. Moreover, we added a maternal history of acquiring toxoplasmosis infection during pregnancy of cases involved in the study and control groups.

Statistical Analysis:

Collected data either through a serological test, questionnaire, or clinical examination were tabulated and subjected to statistical analysis using SPSS (statistical package for social science) on IBM compatible computer, version 20.0 (SPSS Inc., Chicago, IL, USA).

The mean and standard deviation were used to confer quantitative data, while quantitative data were shown as relative and absolute info using confidence intervals (CI) with corresponding 95%. Chi-square test (χ^2) and Fisher's Exact test were used to study the association between qualitative variables, while t-test; is used as a test of significance to compare between two groups that are normally distributed quantitative variables. Odds ratio (OR) is applied as a measurement of risk of certain outcomes to occur related to a specific risk factors. A significant result was considered when P-value was <0.05.

RESULTS

Forty cases were included in the current work, with a mean age of 7.43±2.19, out of the forty participants, 14 were boys (35.0%) and 26 were girls (65.0%). Children living in rural areas represented 12 children (30.0%), compared to 28 children residing in urban areas (70.0%). The most frequent socioeconomic status, was the middle status with 65%, followed by low status with 25%, and lastly high status, represented 10%. Maternal history of previous toxoplasmosis capturing was positive in 27.5% of cases and negative in 72.5% of cases group.

The other 40 children were served as a control group, their mean age was 7.75±2.1. Out of the forty participants, 22 boys (55.0%) and 18 girls (45.0%) were included. Regarding residence, children living in urban areas represented 75.0% (30 children), compared to ten children residing in rural areas (25.0%). The most frequent socioeconomic status, was the middle status with (67.5%), followed by low status with 22.5%, and lastly, the high socioeconomic status, represented 10%. Maternal history of previous toxoplasmosis capturing was positive in 12.5% of cases and negative in 87.5% of cases group. No statistically significant results were observed between general data about age, gender, residence, socioeconomic status and maternal history of toxoplasmosis between healthy and autistic children were presented as shown in table 1.

Table 1: socio-demographic characters among the studied groups

	The studied groups		Test	P value
	Autistic patients N = 40	**Control** N = 40		
Age (years)			t-test	
Mean ±SD	7.43±2.19	7.75±2.11	0.67	0.50
Range	4 – 10	4 – 10		
Gender			X2	
Boys	14 (35.0%)	22 (55.0%)	3.23	0.07
Girls	26 (65.0%)	18 (45.0%)		
Residence				
Rural	12 (30.0%)	10 (25.0%)	0.25	0.62
Urban	28 (70.0%)	30 (75.0%)		
Socioeconomic status				
Low	10 (25.0%)	9 (22.5%)	0.07	0.97
Middle	26 (65.0%)	27 (67.5%)		
High	4 (10.0%)	4 (10.0%%)		
Maternal history of capturing toxoplasmosis				
Positive	11 (27.5%)	5 (12.5%)	2.81	0.09
Negative	29 (72.5%)	35 (87.5%)		

The presence of anti-*Toxoplasma* antibodies in the studied groups was evaluated, seven children (17.5%) having autism were positive for *Toxoplasma* IgG antibodies whereas, 33 autistic children were negative (82.5%). Out of the control group, two children (5%) showed positivity against *Toxoplasma* IgG antibodies in their serum samples whereas, 38 (95.0%) were negative. P-value was 0.15 and no statistically significant difference was recorded (table 2). Anti-*Toxoplasma* IgM antibodies were negative in all examined children.

Table 2: Relationship of toxoplasmosis between autistic patients and their control.

	The studied groups		Test (P value)	Odds ratio	95% CI
	Autistic patients N = 40	**Control N = 40**			
Toxoplasmosis			Fisher's		
Positive	7 (17.5%)	2 (5.0%)	3.13 (0.15)	4.03	0.78 – 20.76
Negative	33 (82.5%)	38 (95.0%)			

Fisher's = Fisher's Exact test

The mean age for children having autism and infected with toxoplasmosis was 9.29±1.49, while in the mean age for children with autism and negative for toxoplasmosis was 7.03±2.13. The difference between the studied groups was statistically significant P-value was 0.006 (table 3).

Table 3: Relationship between age of autistic patients and affection by *Toxoplasma gondii.*

	Age of autistic patients	Range	t-test	(P value)
	Mean± SD			
Toxoplasmosis				
Positive	9.29±1.49	6 -10	3.34	0.006*
Negative	7.03±2.13	4 – 10		

*= P value is significant

Regarding gender in autistic children, three boys (9.1%) out of 33 were positive to toxoplasmosis infection whereas, 30 boys out of 33 (90.9%) were negative. On the other side, four girls (57.1%) out of seven showed positivity to infection with toxoplasmosis whereas, three girls (42.9%) were negative. Percent of positive cases was higher in girls (57.1%) than boys (9.1%) with a statistically significant difference recorded by using Fisher's test for data analysis (table 4).

Table 4: Relationship between gender of autistic patients and affection by *Toxoplasma gondii*.

	Gender of the studied patients		Test (P value)	Odds ratio	95% CI
	Boys N = 33	**Girls N = 7**			
Toxoplasmosis			Fisher's		
Positive	3 (9.1%)	4 (57.1%)	9.24 (0.01*)	13.33	1.97 – 90.07
Negative	30 (90.9%)	3 (42.9%)			

*= P value is significant

Regarding residence, twelve cases were residents in rural areas and 28 came from urban regions. Two autistic children out of twelve, coming from rural areas, had anti- *Toxoplasma* IgG antibodies in their serum samples compared to ten children (83.3%) of the same group who were negative to infection. On the other side, five children (17.9%) out of 28 residing urban areas were positive for toxoplasmosis infection compared to 23 (82.1%). Autistic cases and controls in urban and rural areas were compared in relation to toxoplasmosis capture and the result was not statistically significant (P=1.0) (table 5).

Table 5: Relationship between residence of autistic patients and affection by *Toxoplasma gondii.*

	Residence among the studied patients		Test (P-value)	Odds ratio	95% CI
	Rural N = 12	**Urban N = 28**			
Toxoplasmosis			F		
Positive	2 (16.7%)	5 (17.9%)	9.24 (1.0)	0.92	0.15 – 5.57
Negative	10 (83.3%)	23 (82.1%)			

DISCUSSION

More than three billion human hosts are estimated to be affected with the protozoan parasite *Toxoplasma gondii*[18]. Ihara et al.[19] and Wohlfert et al.[20], *noticed that* changes in the behavior of mice chronically infected with *Toxoplasma gondii* were accompanied by variation in the level of the neurotransmitter, alternation in host immune reaction, and expression of some proteins. The way by which *toxoplasmosis* affects behavior still unknown despite of the past studies[21]. Considering, deficiency of information about the association between toxoplasmosis and autism, which appears to be increasing lately, we performed the current study; aiming to establish the prevalence of *Toxoplasma* antibodies in autistic children in Shebin Al-Kom district, Menoufia Governorate, Egypt. To our knowledge, this is the first paper to be performed in Menoufia Governorate concerning this association.

In the current work prevalence of toxoplasmosis, detected by anti-*Toxoplasma* IgG antibodies, in autistic children was 17.5% (7 cases) compared to 5% (2 children) in the control group which was statistically non-significant (P=0.15), despite the presence of an observed difference between the two groups. The documented risk was three and a half times higher in positive cases and controls. In our study, antibodies to *Toxoplasma* IgM was negative in all studied children. Esnafoglu *et al.*[22], in a similar result, diagnosed three Autism Spectrum Disorder children out of 102 (2.9%) as being associated with anti-*Toxoplasma* IgG, and one control child (2%) out of 51 children, all participants in their study were IgM negative, this result assumes that the role of primary infection or reactive toxoplasmosis in autism pathogenesis don't exist.

The age of children who participated in the study ranged from four to ten years, where positive autistic children with toxoplasmosis infection were 9.29±1.49 (6-10years) and those negative to toxoplasmosis infection were 7.03±2.13 (4-10years). analysis of data showed significant difference with P-value 0.006. Prevention[23] in their report, found that ASD was higher in children aged 4-10 years which may be attributed to family recognition of delay in their children development especially speech. However, ASD can be recognized in earlier stages of disease.

A significant difference was found in autism cases when gender was compared. Boys represented 9.1% of the studied population while girls accounted for 57.1%. with a ratio of 6.2:1 (girls to boys) (P=0.006). This difference may be referred probably to many causes non- representative population is considered one of them. Some parents didn't approve to include their children in the study. This ratio was in contrast to the

ratio reported in previous studies[24] where boys were higher than girls with a ratio of 1:4 - 1:5 (females to males). This may be explained by the hypothesis of higher genetic threshold in females which decrease the risk of getting the disease[25]. In the current study, boys number was higher than girls involved (33 boys and 7 girls), which may be attributed to the ability of girls to cover difficulties in their behavior, in some regions parents, put in mind boys development as a priority more than girls and at the same time, difficulties in females children may be covered in certain societies for traditional habits purposes[26].

History of maternal infection with toxoplasmosis was taken, the study revealed that eleven mothers had a positive history in the autistic group (27.5%), compared to five mothers only in the control group (12.5%) with P-value 0.09, which is a non-significant statistically. However it arises alarm for the risk of congenital infection on the newborn since the ratio between cases and control was 2.2:1. Peyron *et al*.[27] in their study on congenital toxoplasmosis, revealed that fetal affection by toxoplasmosis ranged from asymptomatic presentation to severe variation in the neurological manifestation that may continue throughout life.

The level of socioeconomic status was studied herein, the middle status represented the highest section in all children, it was 65.5% in autistic cases and 67.5% in control group with absence of significant difference (P=0.97). This result agrees with Elbahaaey *et al*.[28] finding, where 72.5% of studied cases were distributed in the middle socioeconomic status.

Regarding residence, our study demonstrated that the prevalence of toxoplasmosis in autistic children in rural area was two cases (16.7%), while the prevalence in urban areas was five cases (17.9%), urban residents were higher than rural ones which may be referred to the elevated level of awareness in population living in urban areas may affect the recognition of the disease in early stages and affect also improvement of symptoms. However, this difference noted between rural and urban was statistically non-significant (P=0.62). Our result was emphasized by Amr *et al.*[29] study, they found that children with autism mostly came from urban regions particularly in Jordan and Egypt.

From the current work, we concluded that despite the absence of significant difference between *T. gondii* infection and autiam, children exhibiting autism disorder are at higher risk of infection with toxoplasmosis. Positive maternal history of toxoplasmosis also icreased the risk of ASD. Therefore, carefull prenatal mangement of toxoplasmosis should be concerned and further research about the pathodenesis of toxoplasmosis in autism children should be considered.

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- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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