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Seroprevalence of toxoplasmosis among children with autism Spectrum Disorder in Mosul, Iraq

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Abstract
Toxoplasmosis is regarded as one of the prevalent challenges in global health caused by parasite *Toxoplasma gondii.* Autism may be a single diagnosis, but the characteristics of autistic people are highly heterogeneous, include phenotypic traits, predictive factors, and biomarkers that contribute to the neurodiversity of autism, which include a variety of medical, social, ecological, and Indigenous models (and their combinations) of disability and difference. Autism The phenomenon known as Paraphraser has been recently associated with a variety of neuropsychiatric and behavioral conditions, with a particular focus on Autism Spectrum Disorder (ASD). The objective of this research endeavor was to assess the prevalence of Toxoplasmosis antibodies in children diagnosed with ASD. **Method:** The investigation was carried out on 154 kids aged between 2 and 12 years, who were divided into two distinct groups. Group 1 comprised 94 children diagnosed with Autism Spectrum Disorder (ASD), while Group 2, serving as the control group, consisted of 60 healthy children. The diagnosis of ASD in the patient group was based on the criteria outlined in the DSM (Diagnostic and Statistical Manual of Mental Disorders). Each child partaking in the study underwent a thorough medical history assessment, a clinical test, and labs tests to identify serum anti-Toxoplasma IgM antibodies utilizing ELISA IgM. Furthermore, the patient group underwent additional evaluation using the Childhood Autism Rating Scale to assess the strong of their symptoms. **Results:** The prevalence of IgM in children with ASD was found to be statistically insignificant when compared to that in typically developing children. There was no significant association observed between Toxoplasma infection and the strong of autistic symptoms in the ASD cohort. Moreover, the investigation indicated a higher level of anti-Toxoplasma IgM antibodies in ASD children with a family history good of this disorder as opposed to those without such a history. Additionally, a higher distribution of anti-Toxoplasma antibodies was noted in children from low socioeconomic backgrounds in contrast to those from moderate or high socioeconomic backgrounds. The aim of the present study was to determine the seroprevalence of T. gondii infection in children with ASD in order to establish a potential link between toxoplasmosis and autism in children. **Conclusions:** The research findings indicated that past exposure to Toxoplasma in children, as opposed to recent exposure, might be associated with the development of Autism Spectrum Disorder (ASD). The Broader Autism Phenotype challenges could also be supported by biological studies of autism that include broader perspectives and topics, as well as enhanced communication and resources. The term “risk factor” is defined as a factor that heightens the likelihood of disease onset, the classification of autism as a disorder remains a topic of contention. Limited terminology options exist for the notion of "risk factors" in facilitating scientific comprehension of the origins, processes, or prognostic indicators of autistic characteristics within human cohorts. This factor may include many factors such microorganism such as parasites like Toxoplasma gondii, is obligate intercellular parasite that transport to the fetal from mother or by feed raw meat or contaminated food.

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Introduction
Autism is classified as a "spectrum disorder," The term "paraphraser" denotes a variation in the severity of symptoms displayed by a child. Autism spectrum disorder (ASD) comprises a collection of intricate neurodevelopmental conditions.

Initial signs may include difficulties in social engagement, as well as verbal and non-verbal expression, as well as repetitive behaviors, are indicative traits of Autism Spectrum Disorder (ASD). Various factors, including levels of fetal testosterone, environmental influences, immunological disparities, obstetric complications, intrauterine infections, genetic predispositions, and gender disparities (with males exhibiting a higher prevalence of autism compared to females), have been associated with the causation of ASD [1]. A youngster with autism spectrum disorder may encounter challenges in effectively communicating and interacting with peers. This can manifest in the form of engaging in repetitive behaviors and movements, exhibiting frustration when faced with disruptions to their daily schedule, and displaying atypical responses to specific circumstances on occasion [2].

Toxoplasmosis is caused by *Toxoplasma gondii* Parasite Considered an epidemic disease that spreads widely worldwide, maternal infection in humans is estimated to affect between 30% and 50% of the global population [3]. The neonate acquires a significant portion of its IgM [4] antibody from the mother, making it a reliable indicator for maternal IgM levels. A study in Turkey revealed a Toxoplasmosis IgM positivity rate of 2.9% in the ASD group and 2% in the control group [5]. The genes of the T. Gondii interactome showed a notable enrichment in the databases of susceptibility genes related to various diseases [6]. Fetal toxoplasmosis infection disrupts the genes involved in the electron transport chain (specifically complexes I and III), which are crucial for the generation of free radicals and the production of oxidative stress. This interference contributes significantly to the pathophysiology of neurodevelopmental and neurodegenerative disorders like autism [7]. The majority of infected neonates are asymptomatic at birth but suffer vision problems later [8]. Symptoms of autistic disorder and indicators of development manifest. Preceding their offspring's initial year of life, caregivers have noted developmental apprehensions; however, as the child reaches two years of age, a significant proportion of caregivers express worries regarding linguistic progression and interpersonal engagement [9]. *Toxoplasma gondi*i is Prevalence of the infection is widespread across various regions globally, reaching reported rates as high as 75%. Gender does not appear to significantly influence prevalence rates as individual's age. Moreover, the infection shows higher prevalence in environments characterized by high temperatures and humidity [10]. During the initial trimester of existence, premature neonates afflicted with toxoplasmosis could manifest central nervous system and ocular afflictions. Conversely, full-term neonates infected with T. gondii typically exhibit a less severe malady, characterized by hepatosplenomegaly and lymphadenopathy within the initial two months post-birth. Upon undergoing routine evaluations for newborns, a substantial proportion of the latter cohort-up to 80%-might subsequently experience cognitive or visual impairments.

[11] Three primary classifications of Toxoplasma gondii genotypes exist, namely type I, II, and III. It is noteworthy that the Type II genotype predominantly contributes to the majority of congenital toxoplasmosis cases [12].

This study was aim to Investigate the epidermology as a means of determining of Toxoplasmosis in children with ASD.

Material and Methods: Materials and Methods

A study involving (154) participants, (94) of whom were children with autism and (60) were healthy children between the ages of (2– 12) years, was conducted in several Hospitals in Mosul /Iraq, from November 2023 to January 2024. Blood samples were taken from the children to test for Toxoplasma IgM levels using the ELISA technique.

Sampling: Initially, Ten ml of blood samples from (154) children (94 atistic patients and 60control) using a sterile syringe were taken.

Detection using ELISA Technique

Detection of Toxoplasma IgM using ELISA technique was performed by measuring anti- Toxoplasma IgG and IgM antibody levels using the Sandwich-ELISA method. The run utilizing the SunLong Biotech. A purified toxo antigen was attached to a microplate according to the manufacturer's instructions in the laboratory. Samples were added to the plate wells and interacted with specific antibodies. Excess components were removed, and a substrate solution was applied, causing the wells containing toxo to change color from blue to yellow. The absorbance was measured at 450 nm, with higher values indicating higher concentrations of toxoplasma antigen. Concentrations were calculated by comparing the sample's absorbance to a standard curve, which allowed researchers to determine specific antibody levels in the serum.

Statistical Analysis: Descriptive statistics, as quantitative variables were described using mean and S.D. for normal distribution and median Qualitative variables were reported as numbers (percentages). Mann-Whitney and Kruskal-Wallis tests assessed IgG and IgM mean difference, false positive (+ve), and negative value, (-ve) values were calculated, determining accuracy indices (positive predictive value, specificity, sensitivity

Result and Discussion

In the present study we enrolled (154) children, (94) with ASD and (60) with non- ASD as healthy control. The positive result was distribution in all ages, the cases with infection in male was higher than in female the age group for infection in males (2 – 4) years the S.D. was 0.178, in (5 – 8) years the S.D. was = 0.289 and in (9 – 12) years the S.D. was= 0.331 while in (2-4) years female the S.D. was= 0.424, in 5 – 8 years the S.D. was = 0.424 and in 9 – 12 years was. D. = 0.303 while the P value was 0.0005 **. The mean in males were 5.980 and in females were 7.179 of autistic cases as, while the P value was 0.045 as in Table 1 and 2.there was no significant difference between the values. The sepisifity was = 2.083 % and Sensefity was = 45.349 % as in Table 3.

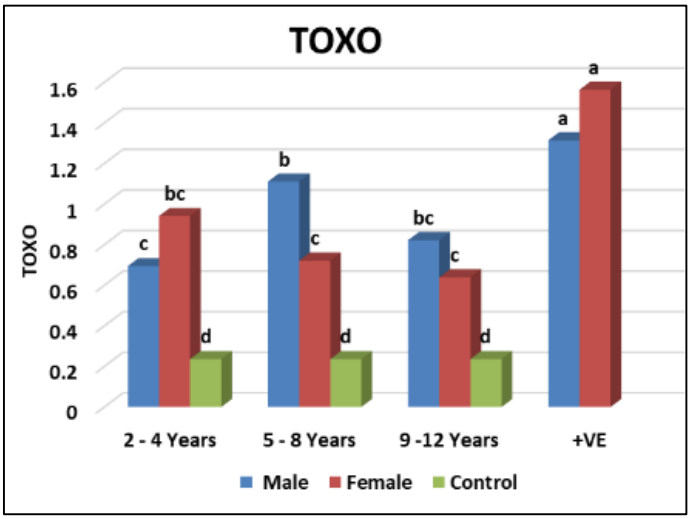

Fig 1: Distribution the Toxoplasmosis on autistic and control children According to Age Group in years

Table1: The Mean ± S.D of age group (years) of children with autism and control under study

Groups	No.	- VE Mean ± S.D.	+ VE Mean ± S.D.
Males	54	6.293 ± 2.791	5.980 ± 2.417
Females	40	8.090 ± 2.528	7.179 ± 2.812
Control	60	-	
P-Value		0.045 *	

Table 2: Mean ± S.D. of Seropsitivity against *Toxoplasma gondii* According to Age (Years)

Groups	AGE (Years)	No. –VE (94)	- VE Mean ± St.d	No. +VE (40)	+ VE Mean ± S.D.
Males	2 – 4	17	0.694 ± 0.169 e	8	1.314 ± 0.178 c
	5 – 8	29	1.111 ± 0.316 d	12	1.563 ± 0.289 b
	9 – 12	12	0.822 ± 0.211 de	6	1.586 ± 0.331 b
Females	2 – 4	4	0.941 ± 0.802 de	2	1.602 ± 0.424 ab
	5 – 8	14	0.721 ± 0.277 e	7	1.642 ± 0.199 ab
	9 – 12	22	0.639 ± 0.140 e	5	1.737 ± 0.303 a
control		60	0.236 ± 0.074 f	-	
P-Value		0.0005 **			

The same letters mean no difference between them

The dissimilar letters indicate that there is no significant difference between the groups for the age groups.

Table 3: Sensitivity and specificity of ELIZA Technique under Study

Sensifity	45.349 %
SpecIfIcIty	2.083 %

An undeniable correlation exists between toxoplasmosis and various mental disorders such as depression, schizophrenia, and autism. Toxoplasmosis is a prevalent parasitic illness that affects humans as well as other endothermic organisms. (13) Studies have indicated that there WAS a notable prevalence of Toxoplasmosis infections among immune compromised individuals and pregnant women [14]. In the context of pregnancy, infections, especially during the initial phases, have been associated with neurodevelopmental issues, predominantly Autism Spectrum Disorder ASD [15]. Serum levels of anti-Toxoplasma gondii IgM and IgG were quantified in children with Autism Spectrum Disorder (ASD) through Enzyme-Linked Immunosorbent Assay (ELISA) and were subsequently juxtaposed with those of healthy children within the corresponding age range. The results stem from an extensive meta-analysis that was undertaken. By Nayeri *et al* [16], Toxoplasmosis has been associated with an elevated likelihood of autism, as evidenced by a greater seroprevalence of anti-*Toxoplasma gondii* IgG antibodies in individuals with autism in comparison to control cohorts. Moreover, *Toxoplasma gondii* has been posited as a potential risk element in the onset of autism [17], found a range of epidemiology of toxoplasmosis higher in autistic kids than in healthy individuals. The study conducted by Hamid *et al*. [18] the seroprevalence toxoplasmosis in autistic children and normal kids. Children with autism had big rate of toxoplasmosis [19]. The changes in epidemiology may resultant from variations in mothers' attributes, such as how they manageable their cats [20] their education history, their health full living practices, and their feeding habits, as well as different in climatic conditions (rain, temperature, soil kinds, altitude, and dry weather) [21]. The findings of the presented study regarding a familial history of autism revealed that, when contrasted with children lacking such a history, children diagnosed with autism and possessing a positive familial background exhibited a notably elevated incidence of past toxoplasmosis. Nevertheless, no such marked distinction was observed in autistic kids with recent toxoplasmosis infection. This search in agreement to Baioumy *et al*. [22] who reported a significant contras between the seroprevalence of past and modren toxoplasmosis and a positive family background of other psychiatric disease

which is schizophrenia among their study categories It show that the strong relationship 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 off spring. This could shed insight on the role of latent toxoplasmosis in the etiology of many neuropsychiatric disorders in mothers and their offspring. It is conceivable that toxoplasmosis plays a substantial role in the etiopathogenesis of mental health disorders, as posited by numerous scholars who have delineated diverse pathways by which Toxoplasma gondii parasites may impact the central nervous system. (23) The individual who declared that the impact of Toxoplasma gondii on the development of psychiatric disorders likely results from the immune response of the brain and the secretion of mediators such as interferon-gamma. Moreover, it was hypothesized that Toxoplasma gondii enhances dopamine levels and the activity of parasitic tyrosine hydroxylase, leading to an upsurge in the manifestation of anxiety [24].

Conclusion

According to the we mentioned above, there is a high risk of getting autism after congenital toxoplasmosis but no definite clue was found. There is no definite evidence about the relationship between the toxoplasmosis and incidence of autism but there are many clues that need further investigation in future. 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.

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