Obat: Jurnal Riset Ilmu Farmasi dan Kesehatan
Volume. 3 Nomor. 1 Tahun 2025
e-ISSN : 3031-0148, dan p-ISSN : 3031-013X, Hal. 120-127
DOI: https://doi.org/10.61132/obat.v3i1.972
Available online at: https://journal.arikesi.or.id/index.php/OBAT

Prevalence of Toxoplasmosis Seropositive Antibodies among Children with Autistic Spectrum Disorders (ASD)

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Abstract: *Toxoplasma gondi is a pervasive parasite that causes a variety of diseases both in humans and animals, it infects the neural cells of the central nervous system. There have been previous reports linking this infection with Autism. Autism is a combination of complex neurodevelopmental disorders leading to alteration in social communication. This study was done to detect the frequency of seropositive Toxoplasmosis in Autistic children attending psychiatric units in Azady Teaching Hospital in Kirkuk governorate-Iraq. This case-control study was done on 40 Autistic children (30 male, 10 female) with an average age of 5.4±6 years and 40 healthy children (8 male, 32 female) with the same age range (6.1±2) year, from April 2018- January 2020. Sample collection was done by obtaining four ml of blood from all study group children for the detection of both IgM and IgG Toxoplasma gondi antibodies using the ELISA technique. Chi-square and paired T-tests were used for data analysis. The IgM serology for both Autistic and control children was negative while there was just one positive result of T.gondi IgG in Autistic Children and two positive results in normal children which made the result insignificant(X2=0.45, p-value>0.05). Regarding gender, there was no significant difference in detecting positive results between boys and girls of the study groups (X2=0.45 p value> 0.05). The study also demonstrated that Autistic Parents were statistically older than healthy children of the same age. We couldn't uncover any connection between ASD and toxoplasmosis. The age of parents & family history has a significant impact on the development of ASD.*

Keywords: *Autism, Toxoplasma gondi, Seroprevalence.*

1. INTRODUCTION

Autism spectrum disorder (ASD) is a common and highly heterogeneous group of complex neurodevelopmental disorders characterized by early impairments with social communication and social interaction, restricted interests, and stereotyped patterns of behaviors, both verbal and non-verbal. Various factors (fetal testosterone levels, immunological imbalance, environmental factors, obstetric complications, intrauterine infections, and genetic background) have been implicated in the etiology of ASD.(1). Toxoplasma gondi is a widespread protozoan parasite that infects a wide variety of animals and causing toxoplasmosis which is one of the more common parasite zoonosis worldwide.T.gondi is an obligate intracellular parasite, which is found inside the reticuloendothelial cells, including muscle and intestinal epithelium of the host. In heavy acute infection, the organism can be free in the blood and peritoneal exudates. It may inhabit the host cell's nucleus but usually lives in the cytoplasm (2, 3).

Received: Oktober 30, 2024; Revised: November 30, 2024; Accepted: Desember 27, 2024;
Online Available: Januari 06, 2025;

There is evidence that some prenatal infections in mothers, including toxoplasmosis, might cause neurological problems in offspring, including autism spectrum disorders. However, the relationship between toxoplasma gondii and autism remains unclear (4).

Immunological anomalies involving cytokines (interleukin [IL]-1R, IL-6, IL-8, IL-10, and IL-17), immunoglobulins, aggravation, and cellular actuation have been famous in people with extreme introvertedness. People with ASD illustrate dynamic irritation within the central nervous framework (CNS); Within the after-death brains of people with ASD, a proinflammatory profile uncovered expanded cytokines and enacted neuroglial reactions. Suggesting that future medications, such as directing cytokine discharge amid pregnancy, might halt children from creating abnormal behaviors.(5). Early-life infections with Toxoplasma gondii have an impact on a child's neurodevelopmental stage. Children are therefore particularly vulnerable. The central nervous system (CNS) is the primary site of Toxoplasma infection, and childhood infections can cause neurodegeneration that compromises CNS function. The discovery that children and adolescents who experience academic difficulties have higher levels of Toxoplasma antibodies than those who are developing normally at the same age lends credence to this theory.

(1). Research on the connection between toxoplasmosis and autism spectrum disorder (ASD) has raised curiosity about possible associations between the two conditions. According to some research, a mother's pregnancy-related Toxoplasma gondii infection may have an impact on the neurodevelopment of her unborn child and raise the likelihood that she may acquire ASD. Finding the prevalence of seropositive toxoplasmosis in autistic children attending psychiatric units in Azadi Teaching Hospital in Kirkuk Governorate, Iraq is the goal of this study.

2. SUBJECTS &METHODS

This case-control study was carried out in Azady Teaching Hospital Kirkuk Governorate- Iraq, from April 2018 to January 2020. Ethical permission was taken from a committee of the hospital. The study was performed on 40 Children (30 male and 10 female) diagnosed with autistic spectrum disorder (ADS) attending the psychiatric unit in Azady Teaching Hospital in Kirkuk, the diagnosis of the cases was done by consultant psychiatrists working in the unit based on DSM-5 (diagnostic and statistical Manual5th edition) of the American Psychiatric Association 2013(6). The CAST (Autism Spectrum Test) was performed for all Autistic patients (7). For research purposes, 40 matched apparently healthy (8 male and 32 female) were included in the study.

3. SAMPLE COLLECTION

Four ml of blood were taken under sterile conditions the serum was separated and stored at -10C until the analysis for anti-Toxoplasma gondi IgM and IgG antibodies.

4. ETHICAL CONSIDERATION

The current study was conducted in accordance with the Declaration of Helsinki's ethical guidelines. Every participant gave verbal and analytical consent before samples are taken; the research was done by the University of Kirkuk's College of Medicine ethics committee.

Estimation of serum anti T.gondi (IgG,IgM) anti body:

All serums were tested for (IgG,IgM)anti T.gondi antibodies using ElIZA kit(GD 80) UK Diluted serum specimen (1:100) were incubated for 20 minutes to permit specific antibodies to T.gondi to attach to antigen-coated well. After wiping out free antibodies and another serum constituent, T gondi-specific IgG was observed utilizing rabbit anti-human IgG conjugated to horseradish peroxidase. after 20-minute incubation, unbound conjugates were eliminated by washing, and TMB enzyme substrate was added for 10 minutes. A blue shade appeared if antibodies to T.godi were found. The addition of stop solution gave a yellow color and optical densities of controls, standards, and samples were estimated by utilizing a microplate reader.

1- IgG

Negative specimens: OD < 15 IU/ml OD

Positive specimens: OD >/= 15 IU/ml OD

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□□
□□□□□□□

Negative specimens: OD < 10 U/ml standard OD

Positive specimens: OD >/= 10 U/ml standard OD

5. PSYCHOLOGICAL ASSESSMENTS

A. CAST Test

Childhood Autism spectrum test on children aged 4-11 years, the test was done to assess the severity of autism spectrum disorder, it contains 39 questions (yes, No) each question has a specific score the total score calculated out of 31, the range from 0-15 considered normal and range above 15 means that the child has ASD or related social

communication difficulties (16). The questionnaire was developed by ARC (the Autism Research Center) at the University of Cambridge.

6. STATISTICAL ANALYSIS

The chi-square test was used to analyze the frequency of anti-T. gondi IgM and IgG antibodies for both patient and control Children. T test was used to show the signified difference between the age of the parent of both the Autistic and the control. Children.

7. THE RESULTS

A prospective cross-sectional study included 40 Autistic children (30 male and 10 female) with an average age of 5.4±6 attending the psychiatric unit in the Teaching Hospital, and 40 normal children (8 male and 32 female) in the same age range (6.1±2). In both groups of children IgM antibodies were negative we did not record any positive results, regarding IgG as shown in Table (1) there were 1 (2.5 %) positive result in Autistic Children and 2 (5%) positive in control children that's make the result insignificant on comparison Toxoxoplasma gondi IgG antibody between Autistic and normal Children.

Regarding gender as in Table (2) there was no significant difference in Toxoplasma gondi IgG anti-body between boys and girls in the study group

As indicated in Table (3) there was a significant difference regarding the age of parents between Autistic and control groups.20% of Autistic children were with positive family history of Autism. Table (4

Table (1) Comparison of *Toxoplasma gondi*IgG antibody between Autistic and normal (Control) children

Toxoplasma IgG	Autistic Children	Normal Children	Marginal Row Total
IgG positive	1	2	3
IgG negative	39	38	77
Total	40	40	80 (Grand total)

The X^2= 0.45, p value> 0.05

Table (2) *Toxopasma gondi* IgG antibody in Male and female in both control and Autistic Children

Toxoplasma IgG	Male	Female	Marginal row Total
IgG positive	1	2	3
IgG Negative	41	36	77
Total	42	38	80(Grand total)

The²X=0.42, p value >0.05

Table (3) Comparison between age of the parents between Autistic and control children.

Parameters	Autistic children	Control children	P value
Mother age(year)	33±5.7	29.8±4.66	P<0.05
Father age(year)	37.±7.88	30.66±4.7	P<0.05

Table (4) Sociodemographic of both patient and control group

	ADS Group		Control Group	
Age (Mean±SD)	%	No	%	NO
Gender Male Female	75% 25%	30 10	20% 80%	8 32
Resident Urban Rural	77.5% .22%	31 9	2.5% 97.5%	3 37
Family History of Autism				
Yes	20%	8	2.5%	1
No	80%	32	97.5%	39

8. DISCUSSION

The current understanding of the association between Toxoplasmosis and autism disorder is complex and somewhat inconclusive. While some studies suggest a potential link between Toxoplasma gondii infection and autism spectrum disorder (ASD), others find no significant association. This case-control study involved 40 Autistic Children with 40 healthy children with almost the same age range. The results obtained from the study showed that there were no statistical differences of both IgG and IgM between Autistic children and their control group ($X2 = 0.45$ $p>0.05$), this result is consistent with a study conducted in Egypt they found no significant difference in the prevalence of Toxoplasma infection between autistic and control children, nor any impact on the Childhood Autism Rating Scale (CARS) score, suggesting no direct association between Toxoplasmosis and autism(4, 8, 9). Afsharpaiam et al 2017in Iran also obtained the same result(10). Another study using a large Finnish birth cohort found that high maternal T. gondii IgM antibodies were associated with decreased odds of childhood autism, while low IgG antibodies were linked to increased odds, indicating a complex relationship that may involve immune response rather than direct causation (4).

The reason for the low incidence of toxoplasmosis among Autistic& control children in our study has different explanations, one of them is the small size of the study, which is not adequate enough to support the hypothesis of the relation of toxoplasmosis in ASD pathogenesis as in other studies. The other explanation is that the climate may affect the

geographical distribution of the parasite, it increases more in hot climates(11). The habit of the population in dealing with animals like cats which is less in Kirkuk city inhabitation than other people living in rural and sub rural areas(12, 13), also this may be return to the fact that approximately one-third of world population is latently infected with T.gondi (ranging between 10 and 80% in different part of the world (without any clinical symptoms (14, 15). The results of our study and other studies (10) are consistent and support the notion that ASD has many etiological factors mostly genetic, but environmental factors for example infections like toxoplasmosis, viral infection and immunization for instance exposure to MMR (measles-mumps-- rubella) immunization might be causing sporadic cases of ASD(16). Moreover, researchers have employed various statistical and modeling techniques to analyze these correlations, similar to those used in climate-related studies of COVID-19. These methods, such as correlation analyses and generalized linear models, help to understand the relationship between toxoplasmosis exposure and developmental issues. However, the findings have not been informative, suggesting that other environmental and genetic factors may also play a significant role in the onset of autism.

As we consider the implications of these studies for public health, it becomes essential to recognize the need for further research. Investigating the interplay between maternal health, environmental factors, and the prevalence of toxic parasites can help clarify the risks involved.

Parental age at the time of conception has been identified as a significant factor influencing the likelihood of autism spectrum disorder (ASD) in offspring, with advanced parental age emerging as a prominent risk factor. In the current study, the ages of parents of children with ASD were notably higher compared to those in the control group, reflecting a global trend. Research consistently demonstrates that both maternal and paternal ages are associated with an elevated risk of ASD, although the underlying mechanisms driving this association remain incompletely understood (17-20). The effects of paternal aging on the sperm epigenome may be at least partially responsible for the increased risk of autism spectrum disorder (ASD) and other neurodevelopmental disorders in children born to elderly fathers. The protein known as brain-enriched guanylate kinase-associated (BEGAIN) is involved in synaptic transmission and protein-protein interactions. A paternal age effect on sperm BEGAIN methylation has been found by a number of epigenome-wide methylation screens(19).

When recording the Sociodemographic data we observe that 20% of the autistic children were with positive family history , many studies go online with this result, Marin et al 2023 demonstrate that dysregulation of DNA methylation is prevalent in ASD, with studies identifying numerous differentially methylated genes associated with the disorder (21). This process can lead to parent-of-origin-specific gene expression, where either the maternal or paternal allele is silenced, affecting neurodevelopmental outcomes(22).

9. CONCLUSION

Latent toxoplasmosis is present in normal children. We did not find a link between Toxoplasmosis & ASD. Family history and age of parents have a great effect on the development of Toxoplasmosis.

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