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The role maternal toxoplasmosis in the development of autism disease in Al-Diwaniyah governorate, Iraq

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***Abstract*---**The study aimed to investigate the role of *Toxoplasma gondii* in causing autism and included 100 autistic patients from different governorates for the period from January to June of the year 2022. The results of the serological analysis showed that 8 of them were infected with toxoplasmosis by 8%, The results of the statistical analysis showed that there were no clear significant differences between the number of autistic patients with toxoplasmosis and their areas of residence at the level of probability (P > 0.05). Genetic variation (mutation analysis) was found in human mitochondrial CoxI gene among autistic patients, NCBI reference samples from one or two mutations, and the total percentage of genetic variation ranged from 2.08-1.04% , while genetic variation (mutation analysis) was found in The human mitochondrial gene Nad1 between autistic patients, healthy subjects and NCBI-Genbank reference samples had from one to two mutations in a proportion of total genetic variance that ranged 1.3-2%.

***Keywords*---**autism, Toxoplasmosis, mtDNA, mutation, maternal.

Introduction

The interest in this disease has increased over the past years because of its important medical effects due to its wide spread throughout the world, and its serious effects on humans, especially pregnant women and newborns, as it causes many cases of miscarriage or stillbirth, as *T. gondii* to the fetus through the placenta from the mother during pregnancy, and even if the child is born after the completion of the pregnancy months, it shows serious symptoms such as mental retardation, epilepsy, the retina of the eye and brain, and the enlargement of the skull with fluids, where the fetal head is deformed or may be smaller than

normal size, and these cases represent about 70-90% (Berrebi, 2007 ; Al-Gharibawi and Al-Waaly,2021).

The IDEA has defined autism as a developmental disability that significantly affects verbal communication. Non-verbal and social interaction, and significant symptoms appear before the third year of life, as they negatively affect the child's educational performance and also lead to the child's preoccupation with repetitive activities and stereotyped movements and his resistance to environmental change or change in the daily routine (Ghazal, 2007). It is also defined as an unusual condition in which the child does not establish his relationships with others, and communicates with them very little, and autism is a term that cannot be used in cases where the child refuses to cooperate because of his fear of the unfamiliar surroundings, and children of any level of intelligence can be affected They may be normal, very intelligent or mentally retarded (Nouri, 2011).

Materials and Methods

Autism patients

The study included 100 children with autism who were diagnosed with autism by specialized doctors at the National Autism Center, Child Protection Hospital, Medical City in Baghdad Governorate for the period from January to June of the year 2022. A form was prepared for each patient, which included information on governorate age, and gender, and asked the mothers whether or not they had Toxoplasmosis during pregnancy.

Samples collection

5 ml of venous blood was drawn from autistic patients and their mothers by a specialized nurse using one syringe each. The sample was then divided into two separate tubes, one without EDTA for serological assay and one with EDTA for molecular assay. The tubes were kept at 4°C until being examined.

Serological evaluation

Determination of Serum anti-Toxoplasma IgM and IgG levels were measured by using the chemiluminescence immunoassay method (Roche Cobas e411 analyzer, Roche Diagnostics, Germany).

Molecular evaluation

mtDNA was extracted using blood genomic DNA kit (Geneaid Extraction Kit DNA gSYAN , United States) .The extracted DNA samples were kept frozen at -20^0 c for further use. PCR assay was performed to detect human mitochondrial CoxI gene were autistic patients and healthy control. The primers used are listed in Table 1, and the PCR reaction program was This method was implemented as described by (Gu et al .2013)

Table 1
Primer of human mitochondrion COXI gene

Primer	Sequence (5` -3`)		Product size
Human mitochondrion coxI gene	F	CACGATTCTTTACCTTTCACTTCATC	84bq
	R	TGATCCCGTTTCGTGCAAG	

Results

Only eight out of a total of 100 autistic children were diagnosed with toxoplasmosis, with an infection rate of 8%, distributed among the different governorates. The results of the statistical analysis showed that there were no statistically significant differences between the number of autistic patients with toxoplasmosis and the different governorates, gender, and residence. The results also showed that there were statistically significant differences Between the ages of autistic patients and their infection with the Toxoplasma parasite (Table 1).

Table 2
Demographic characteristics of Autism patient infected with *Toxoplasma gondii*

Characteristics	autism	T. gondii	X^2	P value
age				
2-5	45	1	3.711	0.03005*
6-15	55	7		
Total	100	8		
gender				
Females	33	3	0.08	0.773
Males	67	5		
Total	100	8		
Governorate's				
Baghdad	46	4	8.511	0.48358
Dewaniyah	23	1		
Basrah	5	0		
Babylon	5	2		
Najif	4	0		
Muthana	2	0		
Wasit	3	0		
Salah aldeen	1	0		
Karkok	2	1		
Baladrose	1	0		
Total	100	8		
residence				
Urban	70	5	0.233	0.62931
Rural	30	3		
Total	100	8		

In light of the positive result of the current study, which included the infection of autistic children with the parasite *T. gondii* at ages ranging from (2-15) years, where the result of the IgG test was positive, which means that IgG continues for life, the laboratory diagnosis of the latent and acute Toxoplasma parasite depends on detection For *Toxoplasma gondii* IgM and IgG antibodies (Hajsoleimani *et al.*, 2012). (Pellaux et al. (1998) explained that the IgG antibody appears 2-3 weeks after the appearance of the IgM antibody and reaches its highest levels within 1-2 months and disappears in different proportions and usually remains for life. In newborns, IgG antibodies are derived to It is largely transmitted from the mother and is transmitted across the placenta to the fetus (Simister, 2003), and (Grether *et al.* (2010) showed that high levels of IgG in the first trimester of pregnancy increased the risk of autism significantly more than in the second and fourth trimesters.

This result was in agreement with (Prandota (2010) study that more boys than girls have autism, and there are significant differences between the sex of autistic patients with and without Toxoplasma condyloma. In males, 5 out of 67 people have autism. The reason for the high number of autistic male patients infected with Toxoplasma parasite is due to the high levels of testosterone that may contribute to the causes of autism in males with toxoplasmosis (Abdoli *et al.*, 2014; Hodková *et al.*, 2007). To verify the existence of a relationship between infection with *Toxoplasma gondii* and the occurrence of autism, a control group (children without autism but with toxoplasmosis) was used, and the result of the statistical analysis was that the probability of children being infected with autism is more in those infected with Toxoplasma parasite compared to non-infected children (Table 3).

Table 3
Infection with *Toxoplasma gondii* in autistic and control patients

Patients	*Toxoplasma gondii*		OR
	Infection	Non infection	
Autism	8	92	1.072
No autism	4	56	
Total	100	50	

Our results agreed with a Turkish study that showed a relationship between toxoplasmosis and autism, as IgG and IgM antibodies were examined, and patients using antibiotics were excluded from the study because of the possibility of affecting the seropositivity of Toxoplasma, and the results showed that children with autism were carriers of toxoplasmosis. condia (Esnafoglu et al., 2017). In an Iranian study by (Najmat Hamid et al., 1998), the studied sample included 100 people, 50 of whom were children with autism and 50 of them were normal children aged (3-12) years. The results showed that children with autism were more infected with Toxoplasma gondii. from the other group. The children infected with this parasite were also more aggressive than the uninfected group. As the researcher (Azizi et al. 2019) explained in the Journal of Veterinary Research for 100 samples, which included 50 samples with autism and 50 normal children aged (3-12) years, and concluded that the rate of infection with components of the condyloma among children with autism was much higher than that of non-

affected children, It also found that 18% of children with autism, including 8% of normal children infected with this parasite, are chronically infected. She confirmed that the rate of toxoplasma infection in children with autism could indicate the relationship between toxoplasmosis infection and autism. The result of our current study also agreed with a Saudi study that showed that maternal toxoplasmosis infection may have a role in the development of autism in childhood, as it is linked to a defect in mtDNA and nDNA (AL-Malki et al.,2021). (Spann .2017), reported a relationship between a high level of maternal IgM antibodies and a decrease in the likelihood of a child developing autism; However, a lack of antibodies to Toxoplasma has the opposite effect of increasing the likelihood of developing autism.

Detection of autism-related genes' mutations

Figure (4-1) Electrophoresis of human mitochondrial COX1 gene on agarose gel with a molecular size (84bp) of autistic patients infected with Toxoplasma parasite (8 samples) and healthy control (2 samples).

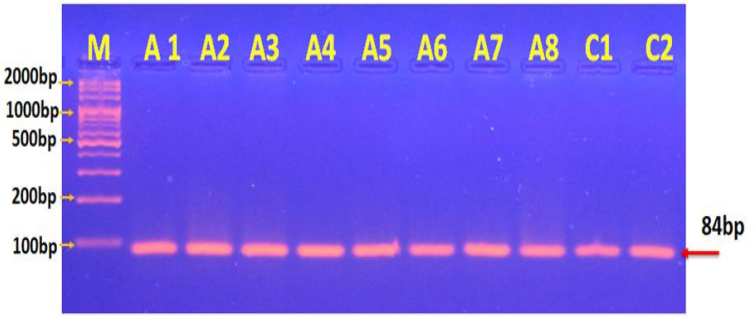

Fig 2. Agarose gel electrophoresis of the COX1 gene for human mitochondria with a size of 84 bp. Where (2000-100) M and samples (A1-A8) are autistic patients, and samples (C1-C2) are healthy subjects

Analysis of genetic variation of the mtCOX1 gene in autistic patients with toxoplasmosis

The DNA sequences of the mtCOX1 gene of autistic patients with and without toxoplasmosis and healthy control samples were shown, the identity of the homologous gene sequence ranged between 97.92 -100%, and genetic changes varied from 0.007-0.002%, and one to two mutations were found and the total percentage ranged for genetic variation 2.08-1.04% (Table 3).

Table 4
Genetic Variance Analysis between Autistic and Healthy Patients

Human mitochondrion CoxI gene Sample	Homology sequence identity (%)			
	No.Mutations	Type of Mutation	Mutation %	Identity %
CoxI autism No.1	2	C/G, T/C	2.08%	97.92%

CoxI autism No.2	1	C/G	1.04%	98.96%
CoxI autism No.3	0	0	0%	100%
CoxI autism No.4	1	A/T	1.04%	98.96%
CoxI autism No.5	0	0	0%	100%
CoxI autism No.6	1	C/G	1.04%	98.96%
CoxI autism No.7	0	0	0%	100%
CoxI autism No.8	1	C/G	1.04%	98.96%
CoxI control No.9	0	0	0%	100%
CoxI control No.10	0	0	0%	100%

Another study revealed that amyloidosis in autism is associated with genetic variation (about 31%) of the COX1 gene (Akouchekian *et al.*,2019). Brain parasitism with *T. gondii* tachyzoites found in various diseases, including neurodegenerative diseases, has all been reported to alter host cell protein stability and degradation, including modulation of brain ATP production by mitochondrial oxidative phosphorylation (Ngo *et el.*, 2017). A previous study(Jamila *et al* (2021) hypothesized that maternal infection with toxoplasmosis from various contaminating sources leads to infection of the fetus via the placenta which is dependent on gestational and quarter gestational infection during infection. Accordingly, fetal toxoplasmosis infection was considered to lead to a weakening of the electron transport chain genes (compounds I and III), which has an important role in the generation of free radicals and the production of oxidative stress, which in turn leads to the pathophysiology of neurodegeneration, neurodevelopment and disorders such as autism.

References

Abdoli A, Dalimi A. Are there any relationships between latent Toxoplasma gondii infection, testosterone elevation, and risk of autism spectrum disorder? Front Behav Neurosci 2014; 8:339.

Akouchekian M, Hakim Shooshtari M, Heidary H, Zahedi Abghari F, Moeinian P. The causative variants of amyloidosis in the autism. Int. J. Neurosci. 2019;129(1):10–5.

Al Maliki Jamilla 5. Al Malk, Nahed Ahmed Hussien and Fuad: Maternal toxoplasmosis and the risk of childhood autism: serological and molecular small-scale studies 2021\ 21:133 https://doi.org/10.1186/s12887-021-02604-4

Al-Gharibawi, Y. K., & Alwaaly, A. B. (2021). Pregnant Women With Toxoplasmosis In Al-Hai City, Wasit Governorate, Iraq. NVEO-NATURAL VOLATILES & ESSENTIAL OILS Journal| NVEO, 5431-5440.

Berrebi, A. B, M. and Bessieres, M (2007): Outcome for children infected with congenital toxoplasmosis in the first trimester and with normal ultrasound finding: a study of 36 cases. Eur. Obst. Gynecol. Rep. Bio. J.135:53-57

Bushra Azizi, Najma Hamid , Hussein Hamidi Najat 2019,Relationship between Toxoplasma gondii contamination and autistic disorder in children Department of Clinical Psychology, College of Educational Sciences and Psychology, Shahid Chamran University of Ahvas, Ahvas, Iran Department of Pathology, College of Veterinary Medicine, Shahid Chamran University of Ahvas, Ahvas, Iran

10.22059 JFR. 2019.252512.2764 Receipt date: April 22, 2010 Acceptance date: July 4, 2010

Esnafoglu,Erman Esra Yancar Demir, Yeliz Cetinkol Mustafa Kerem Calgin Abdullah Erdi Emine Yurdakul Erturk Abdullah Dagli(2017) :The Seroprevalence of Antibodies to Toxoplasma Gondii Among Children with Autism\ Ordu University, Faculty of Medicine, Training and Research\ Dusunen Adam The Journal of Psychiatry and Neurological Sciences 2017,30,309315 DOL 10:5350/DAJPN2017300404

Ghazal Magdy Fath. (2007). Active in a skills development training program Sociology of a type of self-care for autistic children in the city of Amman. Master's thesis. University of Jordan publication.

Grether JK, Croen LA, Anderson MC, Nelson KB, Yolken RH. Neonatally measured immunoglobulins and risk of autism. Autism Res.2010;3(6):323– 32. https://doi.org/10.1002/aur.160.

Gu F, Chauhan V, Kaur K, Brown WT, La Fauci G, Wegiel J, et al. Alterations in mitochondrial DNA copy number and the activities of electron transport chain complexes and pyruvate dehydrogenase in the frontal cortex from subjects with autism. Transl Psychiatry. 2013; 3:3:e299.

Hajsoleimani F, Atarian A, Nourian A. Mazloomzades S. Seroprevalence of Toxoplasma gondii in pregnant women and bioassay of IgM positive cases in Zanjan, Northwest of Iran Iran J Parasitol. 2012;7(2):82-6. PMID:23109950

Hodková H, Kolbeková P, Skallová A, Lindová J, Flegr J. Higher perceived dominance in Toxoplasma infected men- a new evidence for role of increased level of testosterone in toxoplasmosis-associated changes in human behavior. Neuro Endocrinol Lett 2007; 28:110-114.

Jamila S. Al Malki1, Nahed Ahmed Hussien1* and Fuad Al Malki2 : Maternal toxoplasmosis and the risk of childhood autism: serological and molecular small-scale studies . Malki et al. BMC Pediatrics (2021) 21:133 https://doi.org/10.1186/s12887-021-02604-4

Najmat Hamid, Bushra Azizi, Hussein Hamidi Najat 2019. Investigation of Toxoplasma gondii parasite infection and aggression in children with autistic disorder and normal Associate Professor, Department of Clinical Psychology, College of Psychology and Educational Sciences, Shahid Chamran University of Ahvas, Ahvas, Iran Department of Pathology, College of Veterinary Medicine, Shahid Chamran University of Ahvas, Ahvas, Iran Research article (original article Volume 8 Issue 3 Summer, pp. 249 to 262 Accepted Date: 05/31/99 Received Date: 10/12/98

Ngô HM, Zhou Y, Lorenzi H, Wang K, Kim TK, Zhou Y, et al. Toxoplasma Modulates Signature Pathways of Human Epilepsy, Neurodegeneration & Cancer. Sci Rep. 2017;7(1):11496.

Nouri Al-Qamsh Al-Mustafa: Multiple Disabilities. The first edition, Dar Al Masirah for Publishing and Distribution, Amman / Jordan 2011

Pelloux, H.; Brun, E.; Vernet. G.; Marcillat. S. and Ambroise-Thomas, P. (1998). Determination of anti- Toxoplasma gondii immunoglobulin G avidity. Diagn. Microbiol. Infect. Dis., V. (32): Pp.69-73

Prandota J. Autism spectrum disorders may be due to cerebral toxoplasmosis associated with chronic neuroinflammation causing persistent hypercytokinemia that resulted in an increased lipid peroxidation, oxidative stress, and depressed metabolism of endogenous and exogenous substances. Res Autism Spectr Disord. 2010;4(2):119–55.

Simister, N. E. (2003). Placental transport of immunoglobulin G. Vaccine 21, 3365-3369. doi: 10.1016/S0264-410X(03)00334-7.

Spann MN, Sourander A, Surcel HM, Hinkka-Yli-Salomäki S, Brown AS. Prenatal toxoplasmosis antibody and childhood autism. AutismRes.2017;10(5):769–77. https://doi.org/10.1002 /aur.1722.