Original Article

Toxoplasmosis among children with Down syndrome: A case-control study

Ayat A Elblihy[1], Amira I Taman[1], Yahya M Wahba[2], Marwa M Hamouda[1]

Departments of Medical Parasitology[1] and Pediatrics[2], Mansoura University, Faculty of Medicine, Mansoura, Egypt

ABSTRACT

Background: Abnormal folate metabolism might predispose to neural tube defects and Down syndrome (DS). *T. gondii* employs folates for its biosynthetic processes and has been implicated as a cause of altered folate levels, which may be a predisposing factor for DS.
Objective: As little is known about the association between toxoplasmosis and DS, we aimed to assess the predisposing factors, and potential risk factors of toxoplasmosis and relation to folate metabolism among children with DS.
Subjects and Methods: A case-control study was conducted including 90 children with DS, 90 healthy controls, and their mothers. They were investigated for anti-*T. gondii* IgG antibodies and serum folic acid levels. A questioner was also formulated to investigate the potential risk factor for each participant.
Results: Anti-*T. gondii* IgG antibodies were significantly detected in 17.78% of children with DS and 6.67% of controls (P=0.023). Exposure to toxoplasmosis risk factors such as contact with the farm animals, soil exposure and drinking raw milk were more frequent among mothers of DS children. Serum folic acid levels were lower in mothers of DS children (53.3%) than mothers of the control group (33.3%), and in toxoplasmosis positive-DS children than toxoplasmosis negative-DS children.
Conclusion: Toxoplasmosis appeared to be more frequent among DS children with major contributing effects of the socio-demographic factors. Maternal folate deficiency is apparently associated with toxoplasmosis in children with DS.

Keywords: children; Down syndrome; folic acid; maternal; toxoplasmosis.

Received: 21 May, 2023; **Accepted:** 6 August, 2023.

Corresponding Author: Ayat A. Elblihy**; Tel.:** +20 40 2360187**; Email:** dr_ayat@mans.edu.eg

Print ISSN: 1687-7942, **Online ISSN:** 2090-2646, **Vol. 16, No. 2, August, 2023.**

INTRODUCTION

Toxoplasmosis is a cosmopolitan parasitic infection caused by the apicomplexan *T. gondii*. Felines are the only definitive host for *T. gondii*[1]. However, it is transmissible to humans and other vertebrates by several means involving ingestion of tissue cysts in undercooked meat, drinking water contaminated by oocysts, contact with oocysts deposited in soil by cats, and congenitally from mother infected during pregnancy to her fetus. In human congenital toxoplasmosis has been associated with long-term neurodevelopmental disorders[2-3] indicating a clear link between toxoplasmosis and neurological conditions. In verification anti *T. gondii* antibodies were detected in patients sera with neurological disabilities such as autism, schizophrenia and cerebral palsy[4-6].

Besides, folic acid is crucial for the de novo building up of nucleotide precursors, where folate is provided by diet or supplemented as a drug, since humans cannot synthesize it[7]. The brain needs a continuous supply of the dietary folate for the production of neurotransmitters, early embryonic neurodevelopment and adult neurogenesis[8]. Accordingly, lower availability of folate was associated with neurological disorders and lower cognitive functions[9].

Besides deficiency due to possible insufficient folate intake or inefficient metabolism due to polymorphisms in folate pathway results in DNA strand breaks, DNA hypomethylation, abnormal gene expression, chromosomes aneuploidy and apoptosis which might predispose to trisomy 21[10]. The role of folic acid deficiency in DS predisposition was confirmed[11,12]. It is established that *T. gondii* could harvest host folate[13] with consequent increase in the homocysteine level in multiple brain regions causing damage of the neural cells and impairment of the cognitive functions[14].

To our knowledge, little is known regarding association of toxoplasmosis among children with DS and the possible links between maternal toxoplasmosis and serum folic acid levels in DS

 DOI: 10.21608/puj.2023.212443.1212

children and their mothers. From this point, we estimated the seroprevalence of the anti-*T. gondii* IgG antibody among children with DS and their mothers, and attempted to shed more light on the possible maternal risk factors, particularly the serum folic acid that could contribute to DS occurrence. We also highlighted the possible risk factors of toxoplasmosis among DS children and their mothers.

SUBJECTS AND METHODS

This case control study was conducted at Medical Parasitology department, Faculty of Medicine, Mansoura University during the period from February 2019 to February 2022.

Study design: The case-control study includes karyotype-proven children with DS (case group), and apparently healthy non-DS children as a control group. Investigation parameters include measurement of anti-*T. gondii* IgG antibodies and serum folic acid levels in both groups. A questioner is formulated to investigate the potential risk factor for each participant.

Study participants: The study included 90 karyotype-proven children with DS (age 2-17 years) who were recruited from the genetic outpatient clinics of Mansoura University Children's Hospital, Egypt. Children with other chromosomal abnormalities, other neurological disorders, neoplastic or autoimmune diseases, or receiving immunosuppressive drugs, and those with possible neurological infection, were excluded from the study. Additionally, 90 apparently healthy non-DS children without any neurological manifestations were enrolled as a control group. The healthy children included those attending general outpatient clinics of the same hospital for minor complaints e.g., pharyngitis or for regular follow-up visits.

Clinical evaluation: All participants were assessed for their socio-demographic characters including gender, age, residence, parental level of education and monthly income. In addition, mothers were assessed for lifestyle habits pertaining to infection such as contact with farm animals, cats, dogs, exposure to soil, consumption of raw milk, and unwashed vegetables. Maternal previous toxoplasmosis and blood transfusions were also recorded.

Laboratory work-up: A blood sample (3 ml) was collected from each participant into a pre-labeled plain tube under aseptic conditions. Sera were stored at -20°C, and tested for anti-*Toxoplasma* IgG antibodies using commercial ELISA kits (SERION ELISA classic, Friedrich-Berrgius, Wurzburg, Germany)[15]. According to the manufacturer's instruction, its specificity and sensitivity were 99.4% and 98.2%, respectively. Positive and negative control sera were included in each plate to ensure the integrity of the reagents and technical procedures. Folate levels (normal: 4.6-34.8 ng/ml) in the sera of participants, and their mothers were measured using the commercial kit (Elecsys Folate III, Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions[16].

Statistical analysis: SPSS version 25 was used for data analysis. Qualitative variables were presented as number (percent). For continuous variables, the data were tested for normality with Kolmogorov-Smirnov test. Non-parametric data were presented as median (minimum-maximum) while parametric data were presented as means and standard deviations. Univariate and multivariate analyses were carried out for the risk factors associated with DS occurrence. One way analysis of variance (ANOVA) test with the least significant difference (LSD) post hoc multiple comparisons was used for between-group comparison. $P \leq 0.05$ was considered statistically significant.

Ethics consideration: The study procedures were approved by the Institutional Research Board of Mansoura University Faculty of Medicine, Egypt (Code number: R.19.04.475), and conducted in accordance with the 1964 Helsinki declaration, and its later amendments. Written informed consent was obtained from parents and/or legal guardians of the study participants. The study results were reported to the mother of each participant.

RESULTS

Sociodemographic data of the study groups: Table (1) shows that more than half of the DS cases (54.4%) were males compared to 61.1% in the control group. Most of the studied cases who were ≤10 years old (83.3% for DS group, and 94.4% for the control group), lived in rural areas (77.8%) compared to the control group ($P<0.001$). Numbers of educated mothers and fathers were significantly lower among DS cases than the control group ($P= 0.006$ and <0.001, respectively). Low monthly income was more prevalent among DS group than controls ($P<0.001$).

The risk factors for DS occurrence: The logistic regression model revealed that toxoplasmosis was the only independent risk factor for DS occurrence with adjusted odds ratio of 3 (Table 2).

Anti-*Toxoplasma* IgG antibodies among the study groups: The percentage of positive anti-*Toxoplasma* IgG antibodies among DS group was 17.78% (16/90) compared with 6.67% (6/90) among the control group ($P=0.023$). The association between DS and toxoplasmosis is represented in figure (1A) {Odds ratio (95% confidence intervals) = 3 (1.2-8)}. The median of seropositive *Toxoplasma* antibody titer was significantly higher among DS group than the control group ($P= 0.001$) (Fig. 1B).

The risk factors for toxoplasmosis among mothers of study groups: Table (3) shows that the exposure to toxoplasmosis risk factors was significantly higher among mothers of DS group than mothers of the control group regarding the contact with cats (*P*<0.001), farm animals (*P*=0.02), soil exposure (*P*=0.002), consumption of raw milk (*P*<0.001), and low folic acid levels (*P*=0.01). Moreover, previously diagnosed maternal toxoplasmosis was significantly higher (13.3%) among mothers of DS children (*P*<0.001). Exposure of pregnant mothers to farm animals, soil and raw milk consumption increased the risk of bearing DS children {odds ratios = 2.2, 4.0 and 19.5, respectively}.

Serum folate levels among mothers and children: Folate deficiency was more frequent among mothers of DS group (53.3%) than mothers of the control group (33.3%) (*P*=0.01) (Table 3). Folate levels were also lower in children with toxoplasmosis, whether normal or having DS, but this was statistically insignificant. There was a significant association when comparing serum folic acid level in DS children positive for toxoplasmosis (3.8 ng/ml), with children who had DS only (6.2 ng/ml) (*P*=0.001) (Table 4).

Table 1. The sociodemographic characteristics of Down syndrome and control groups.

Characters		DS group (No.=90) No. (%)	Control group (No.=90) No. (%)	Chi-square test *P* value
Gender	**Male**	49 (54.4)	55 (61.1)	
	Female	41 (45.6)	35 (38.9)	0.36
Age (years)	**≤ 10**	75 (83.3)	85 (94.4)	
	> 10	15 (16.7)	5 (5.6)	0.018
	Median (Min-Max)	3 (0.1-17)	5 (1-11)	
Residence	**Rural**	70 (77.8)	28 (31.1)	
	Urban	20 (22.2)	62 (68.9)	<0.001
Maternal education	**Educated**	59 (65.6)	75 (83.3)	
	Illiterate	31 (34.4)	15 (16.7)	0.006
Father education	**Educated**	50 (55.6)	89 (98.9)	
	Illiterate	40 (44.4)	1 (1.1)	<0.001
Monthly income	**Low**	37 (41.1)	17 (18.9)	
	Moderate	52 (57.8)	40 (44.4)	<0.001
	High	1 (1.1)	33 (36.7)	

Table 2. Univariate and multivariate logistic regression analysis of the risk factors for Down syndrome occurrence.

Risk factors	*P* value	COR (95% CI)	AOR (95% CI)
Gender (Male)	0.43	1.5 (0.8-2.1)	
Residence (Rural)	0.004*	1.7 (1.3-2.8)	
Maternal education (Illiterate)	0.013*	1.9 (1.3-4.8)	
Father's education (Illiterate)	0.3	2.1 (0.9-5.8)	
Monthly income (Low)	0.12	2.1 (0.85-6.3)	
Toxoplasmosis	0.002*	2.9 (1.3-7.6)	3 (1.2-8)

COR: Crude odds ratio; **AOR:** Adjusted odds ratio; **CI:** Confidence interval; **":** Significant (*P*≤ 0.05).

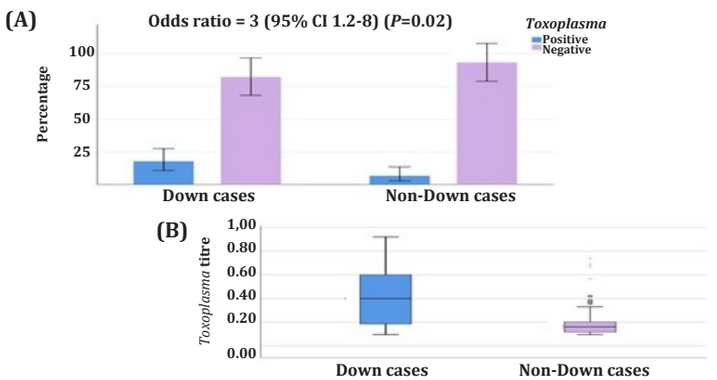

Fig. 1. Percentage of anti-*Toxoplasma* IgG antibodies (A), and titre (B) in the Down syndrome and control groups.

Table 3. Risk factors of toxoplasmosis during pregnancy among mothers of Down syndrome and control groups.

Risk factors		Mothers		Statistical analysis	
		DS No. (%)	Control No. (%)	*P* value	OR (95% CI)
Contact with cats	**Yes**	29 (32.2)	0 (0)		
	No	61 (67.8)	90 (100)	<0.001*	
Contact with dogs	**Yes**	3 (3.3)	0 (0)		
	No	87 (96.7)	90 (100)	0.2	
Contact with farm animals	**Yes**	46 (51.1)	29 (32.2)		
	No (r)	44 (48.9)	61 (67.8)	0.02*	2.2 (1.2-4)
Soil exposure	**Yes**	19 (21.1)	5 (5.6.)		
	No (r)	70 (77.8)	85 (94.4)	0.002*	4.0 (1.6-12.9)
Handling raw meat	**Yes**	33 (36.7)	37 (41.1)		
	No	44 (48.9)	53 (58.9)	0.6	
Consumption of raw milk	**Yes**	59 (65.6)	8 (8.9)		
	No (r)	31 (34.4)	82 (91.1)	<0.001*	19.5 (8.4-45.5)
Eating unwashable vegetables/fruits	**Yes**	2 (2.2)	1 (1.1)		
	No	88 (97.8)	89 (98.9)	0.6	
Washing hands before meals	**Yes**	77 (85.6)	83 (92.2)		
	No	13 (14.4)	7 (7.8)	0.15	
Blood transfusion	**Yes**	33 (36.7)	37 (41.1)		
	No	57 (63.3)	53 (58.9)	0.5	
Previous toxoplasmosis	**Yes**	12 (13.3)	0 (0)		
	No	78 (86.7)	90 (100)	<0.001*	
Folic acid levels	**Normal**	42 (46.7)	60 (66.7)		
	Low	48 (53.3)	30 (33.3)	0.01*	

OR: Odds ratio; **CI:** Confidence interval, ***:** Significant ($P \leq 0.05$).

Table 4. Serum folic acid levels among the Down syndrome and control groups in relation to anti- *Toxoplasma* IgG.

Groups	Folic acid (ng/ml)#	Statistical analysis	
		ANOVA	Bonferroni post-hoc
DS children with			
Positive anti-*Toxoplasma* IgG (n=16)	3.8 ± 1.6		0.001**
Negative anti-*Toxoplasma* IgG (n=74)	6.2 ± 2.1		
Control children with		F=12.4; P=0.001*	
Positive anti-*Toxoplasma* IgG (n=6)	3.21 ± 1.1		0.13
Negative anti-*Toxoplasma* IgG (n=84)	4.3 ± 1.5		0.012**

Data expressed as means ± SD; **#:** Normal folic acid level = 4.6-34.8 ng/ml; **ANOVA:** Analysis of variance; ***:** Significant difference from DS children; ****:** Significant difference from DS children with positive anti-*Toxoplasma* IgG.

DISCUSSION

Data relating toxoplasmosis and folate levels to Down syndrome require interpretation. The current case-control study revealed higher seroprevalence of *Toxoplasma* antibodies among DS cases indicating greater exposure than the controls. This was associated with the sociodemographic characters and the higher exposure to toxoplasmosis risk factors. Notably, most DS children were of rural residence, lower monthly income, and lower paternal education levels than the controls. In confirmation it was reported that the residence area has an indisputable role in increasing the risk of such a parasitic infection[17]; and the lack of urbanized housing markedly increases the likelihood of infection[18]. Also, poverty and low paternal education levels are well-known contributing factors in increasing the risks of toxoplasmosis. This is in accordance with a previous national study that reported higher toxoplasmosis immunoglobulin levels among DS cases than controls[19]. Interestingly, the current study reported a higher seroprevalence among the younger age group (less than ten years). On the contrary, several researches reported higher seroprevalence of toxoplasmosis among the older age groups[18,20,21].

(B)

To our knowledge, no previous studies had evaluated the risk factors of toxoplasmosis among mothers having DS children. The current study found that most of DS cases were from rural areas, and their parents had low income and socioeconomic status. This finding confirms the relationship between the low socioeconomic standards and DS[22,23]. However; Al-Awadi *et al*.[24] reported insignificant differences between peoples living in rural or urban areas. In agreement the current study, found no significant difference among seropositive DS cases and controls regarding the gender.

Regarding the toxoplasmosis risk factors among mothers, contact with cats was more common among

mothers of DS children, since street cats are one of the serious risk factors in rural areas where they traffic easily into farms and houses releasing oocysts outdoors and indoors in their feces. These oocysts sporulate in the soil, and remain infective for months or even years[25]. Our results are in agreement with El-Beshbishi *et al*.[19] who revealed significant associations between *Toxoplasma* seropositivity and the history of close contact with cats, farm animals and soil in children with DS. The current study also indicated that the maternal contact with farm animals, exposure to soil and consumption of raw milk during pregnancy increased the likelihood of having DS child (OR = 2.2, 4.0 and 19.5 times, respectively). This finding to some extend agrees with Minbaeva *et al*.[26].

Evidence for involvement of congenital toxoplasmosis in the pathogenesis of several neurodevelopmental disorders is relatively strong[2-4]. The current study suggested that toxoplasmosis was an independent risk factor for DS occurrence. Awareness of this and other possible risk factors for such genetic disease would eventually reduce the burden of care in their families[27]. The current study recorded higher serological reaction to *T. gondii* among children with DS than healthy cases, which strongly supports El-Beshbishi *et al*.[19] who reported that 13.3% of children with DS were seropositive for *T. gondii* IgG with higher values than controls. Another study reported that 34.5% of children with DS had higher *T. gondii* IgG antibody levels than controls[4], however this higher seroprevalence may be attributed to different sample size, population age, location and test sensitivity. These results suggesting that toxoplasmosis may be involved in the pathogenesis of DS, were further investigated for the possibility of the parasite competing for the host folate in the mothers.

In this context, the current study shows that the higher percentage of mothers having DS children had lower serum folate levels when compared with mothers of normal children. Therefore, the low folate level could be considered as a significant risk factor for giving birth to a baby with DS. Moreover, DS children positive for toxoplasmosis had significantly lower serum folic acid concentrations than *Toxoplasma*-free DS children; this could be explained by the potential effect of toxoplasmosis on host folate level. However, the role of feeding habits in inducing folate deficiency should not be ignored. Hence, intervention strategies including healthy maternal nutrition were preferred and considered for better developmental purposes[28,29].

Dietary folate is an essential B-group vitamin working as a donor and an acceptor of carbon units during the synthesis of DNA, amino acids and S-adenosyl methionine[30]. Folate deficiency was linked to numerous neurodevelopmental disorders, and might be associated with reduced cognitive functions[9]. Additionally, abnormal folate metabolism might predispose to neural tube defects, vascular diseases and DS[31]. Earlier studies suggested a possible link between DS and abnormal folate metabolism[10,11,30]. *Toxoplasma* was implicated as a cause of altered folate levels, and studies reported that *T. gondii* could play a fundamental role in the occurrence of DS through folate deficiency with subsequent chromatin structure damage and host cell cycle dysregulation[32,33].

Our aforementioned results supported that toxoplasmosis in mothers probably predisposed to folic acid deficiency, which might have led to a higher risk of chromosomal abnormalities and consequently DS. In maternal primary infection or congenital latent infection, transplacental transmission of *Toxoplasma* is common with invasion of different fetal tissues causing malformations[34,35].

Although the present study included the limitation of being a single center study with a relatively small sample size, the associations between exposure to toxoplasmosis and DS due to lower maternal folate levels was confirmed. Rural residence, poverty and low paternal education levels were also significantly associated with higher risk of toxoplasmosis exposure among DS group. Further large-scale extended studies are recommended to confirm our findings. For primary prevention of DS, we suggest early screening and treatment of mothers with low serum folic acid level, and/or positive anti-*Toxoplasma* IgG antibodies.

Author contribution: All authors contributed to the study design and conception. Preparation of materials, data collection and analysis were carried out by Wahba YM, Elblihy AA and Taman AI. The laboratory work-up was performed by Elblihy AA and Hamouda MM. The first draft of the manuscript was written by Taman AI and Wahba YM. All authors revised, approved the final manuscript, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work.

Conflict of interest: The authors declare that they have no conflict of interest.

Funding statement: No funds, grants, or other support was received.

REFERENCES

1. Balcha TM, Aga BI, Disasa DD, Berhanu G. Public health and economic significance of toxoplasmosis. Am-Euras J Sci Res 2020; 15:112-121.
2. Khan K, Khan W. Congenital toxoplasmosis: An overview of the neurological and ocular manifestations. Parasitol Int 2018; 67(6):715-721.
3. Spann MN, Sourander A, Surcel H-M, Hinkka-Yli-Salomäki S, Brown AS. Prenatal toxoplasmosis antibody and childhood autism. Autism Res 2017; 10(5):769–777.
4. Shehata AI, Hassanein FI, Abdul-Ghani R. Seroprevalence of *Toxoplasma gondii* infection among patients with non-

schizophrenic neurodevelopmental disorders in Alexandria, Egypt. Acta Trop 2016;154:155–159.
5. Runge K, Balla A, Fiebich BL, Maier SJ, Pankratz B, Schlump A, *et al*. Antibody indices of infectious pathogens from serum and cerebrospinal fluid in patients with schizophrenia spectrum disorders. Fluids Barriers CNS 2022; 19(1):61-73.
6. 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.
7. Shams A. Folates: An introduction. In "B-Complex Vitamins: Sources, Intakes, and Novel Applications. Edited by Leblanc JG. 2022; DOI: 10.5772/intechopen.102349
8. Imbard A, Benoist JF, Blom HJ. Neural tube defects, folic acid and methylation. Int J Environ Res Public Health 2013; 10(9):4352-4389.
9. McGarel C, Pentieva K, Strain JJ, McNulty H. Emerging roles for folate and related B-vitamins in brain health across the lifecycle. Proc Nutr Soc 2015; 74(1):46–55.
10. Enciso M, Sarasa J, Xanthopoulou L, Bristow S, Bowles M, Fragouli E, *et al*. Polymorphisms in the mthfr gene influence embryo viability and the incidence of aneuploidy. Hum Genet 2016; 135:555–568.
11. Jiajin L, Shuyan C, Ying W, Junxiao C, Xiudi W. Genetic polymorphisms in folate metabolism as risk for Down syndrome in the Southern China. J Matern Fetal Neonatal Med 2019; 32(12):2030-2035.
12. Chatterjee M, Saha T, Maitra S, Sinha S, Mukhopadhyay K. Folate system gene variant rs1801394 66a>G may have a causal role in Down syndrome in the Eastern Indian population. Int J Mol Cell Med 2020; 9(3):215-224.
13. Coppedè F. Risk factors for Down syndrome. Arch Toxicol 2016; 90(12):2917–2929.
14. Berrett AN, Gale SD, Erickson LD, Brown BL, Hedges DW. *Toxoplasma gondii* moderates the association between multiple folate-cycle factors and cognitive function in U.S. adults. Nutrients 2017; 9(6):564.
15. Jin S, Chang ZY, Ming X, Min CL, Wei H, Sheng LY, *et al*. Fast dipstick dye immunoassay for detection of immunoglobulin G (IgG) and IgM antibodies of human toxoplasmosis. Clin Vaccine Immunol 2005; 12(1):198-201.
16. Paşca SP, Dronca E, Kaucsár T, Crăciun EC, Endreffy E, Ferencz BK, *et al*. One carbon metabolism disturbances and the C677T mthfr gene polymorphism in children with autism spectrum disorders. J Cell. Mol Med 2009; 13(10):4229-4238.
17. Wang S, Yao Z, Li H, Li P, Wang D, Zhang H, *et al*. Seroprevalence and risk factors of *Toxoplasma gondii* infection in primary school children in Henan province, central China. Parasite 2020; 27:23.
18. Yan C, Liang LJ, Zheng KY, Zhu XQ. Impact of environmental factors on the emergence, transmission and distribution of *Toxoplasma gondii*. Parasites Vectors 2016; 9:1-7.
19. El-Beshbishi SN, Elzeky SM, Atia RA, Abdalaziz KF, El-Tantawy NL. *Toxoplasma gondii* infection among Egyptian children with neurological disorders: developmental and risk factors analysis. PUJ 2020;13(3):190-196.
20. Marchioro AA, Colli CM, Ferreira EC, Viol BM, Araújo SM, Falavigna-Guilherme AL. Risk factors associated with toxoplasmosis and toxocariasis in populations of children from nine cities in southern Brazil. J Helminthol. 2015; 89(4):428–432.
21. Flegr J, Prandota J, Sovičková M, Israili ZH. Toxoplasmosis-a global threat: Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. PloS one 2014; 9(3):e90203.
22. Alam El-Deen N, A Alwakeel A, El-Gilany A-H, Wahba Y. Burden of family caregivers of Down syndrome children: A cross-sectional study. Fam Pract 2021; 38(2):160-165.
23. Hunter JE, Allen EG, Shin M, Bean LJ, Correa A, Druschel C, *et al*. The association of low socioeconomic status and the risk of having a child with Down syndrome: A report from the National Down Syndrome Project. Genet Med 2013; 15(9):698–705.
24. Al-Awadi AA, Abdul-Ghani R, Al-Mekhlafi AM. *Toxoplasma gondii* infection among institutionalized children with Down syndrome in Sana'a city, Yemen: Implications of low IgG seroprevalence. Acta Parasitol 2022; 1:1-5.
25. Taman A, Alhusseiny S. Exposure to toxoplasmosis among the Egyptian population: A systematic review. PUJ 2020; 13(1):1–10.
26. Minbaeva G, Schweiger A, Bodosheva A, Kuttubaev O, Hehl AB, Tanner I, *et al*. *Toxoplasma gondii* infection in Kyrgyzstan: Seroprevalence, risk factor analysis, and estimate of congenital and AIDS-related toxoplasmosis. PLoS Negl Trop Dis 2013; 7(2):e2043.
27. Moretti A, Cianci P, De Paoli A, Meroni F, Tajè S, Mariani M, *et al*. Burden of care in families of patients with rare genetic diseases: Analysis of a large Italian cohort. Eur J Med Genet 2021; 64(7):104230.
28. McCann S, Perapoch Amadó M, Moore SE. The role of iron in brain development: A systematic review. Nutrients 2020; 12(7):2001.
29. Caffarelli C, Santamaria F, Procaccianti M, Piro E, Delle Cave V, Borrelli M, *et al*. Developments in pediatrics in 2020: Choices in allergy, autoinflammatory disorders, critical care, endocrinology, genetics, infectious diseases, microbiota, neonatology, neurology, nutrition, ortopedics, respiratory tract illnesses and rheumatology. Ital J Pediatr 2021; 47(1):232.
30. Kaye AD, Jeha GM, Pham AD, Fuller MC, Lerner ZI, Sibley GT, *et al*. Folic acid supplementation in patients with elevated homocysteine levels. Adv Ther 2020; 37:4149-4164.
31. Coşar A, İpçioğlu OM, Özcan Ö, Gültepe M. Folate and homocysteine metabolisms and their roles in the biochemical basis of neuropsychiatry. Turk J Med Sci 2014; 44(1):1-9.
32. Rahman T, Gulshan JE, Rahman A. Impact of *Toxoplasma gondii* infection on human health. BRC 2022; 8(1):1093-1099.
33. Norins LC. Down syndrome and Alzheimer's disease: Same infectious cause, same preventive? Med Hypotheses 2022; 158: DOI: 10.1016/j.mehy.2021.110745.
34. Mcauley JB. Congenital toxoplasmosis. J Pediatric Infect Dis Soc 2014; 3(1):S30-S35.
35. Puccio G, Cajozzo C, Canduscio LA, Cino L, Romano A, Schimmenti MG, *et al*. Epidemiology of *Toxoplasma* and CMV serology and of GBS colonization in pregnancy and neonatal outcome in a Sicilian population. Ital J Pediatr 2014; 40:23.