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TOXOPLASMOSIS AND NEUROLOGICAL DISEASES IN CHILDREN: A SERIOUS RISK OR MERE ASSOCIATION

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Abstract

Tracing the etiology of paediatric neurological disorders back to toxoplasmosis was often difficult and particularly challenged, depended mainly on serology in common practice. The current study evaluated the role of toxoplasmosis and oxidative stress in Egyptian children suffering from acute neurodevelopmental disorders, by seroprevalence in relation to IFN gamma in patients with positivity for IgG & IgM. The result showed that out of 155 patients, 128 (82.6%) were positive for IgG and 10 (6.5%) were positive for both IgG & IgM. Also, the serum malondialdehyde (MDA) was significantly higher in seropositive pa-tients than in seronegative ones. Six children suffered from acute toxoplasmosis showed high levels of serum IFN gamma.

Key words: Toxoplasmosis, Neurological disorders, Seroprevalence, qPCR, MDA, IFN gamma

Introduction

Toxoplasma gondii (*T. gondii*) is a worldwide protozoon parasitic burden, causing toxoplasmosis (Flegr *et al,* 2014). *T. gondii* has a wide range of hosts including felids as definitive hosts and humans and other warm-blooded animals as intermediate hosts (Bassiony *et al*, 2016). Toxoplasmosis can be transmitted in several ways and is therefore precipitated by a multiplicity of risk factors. Modes of infection include ingestion of tissue cysts which might be present in undercooked meat or dairy products or ingestion of oocysts in unwashed vegetables and fruits. Organ transplantation and blood transfusion have also been reported as potential portals for parasite entry (Flegr, 2013). In addition, congenital toxoplasmosis is caused by the transplacental transmission of tachyzoites (Alharbi *et al*, 2017). Although 30% of exposed fetuses acquire the infection from the mother, only 15 % of the babies showed clinical signs of infection at birth, while the majority would appear clinically normal. The development of clinical disease is determined by several factors, especially the gestational age at which infection occurred, where infection acquired at an early gestational age is known to result in a more severe clinical picture as compared to infection acquired in the second and third trimesters (Hill and Dubey, 2002).

Congenital toxoplasmosis may therefore be asymptomatic, or it may present as clinically severe disease during the neonatal period. It may also take a less severe form which manifests during the early months of life. Finally, relapse of an undiagnosed infection can occur later in life, even as late as in adulthood (Remington *et al*, 2011). The immune status of the host was detrimental to the outcome of toxoplasmosis, where Th1 cytokines such as IFN ga-mma play a pivotal role in the anti-*Toxoplasma* immune response (Gavrilesu and Denkers, 2001).

The neurotoxoplasmosis was explained by several mechanisms such as the disturbance of glutamate homeostasis that interfered with glutamate transport (David *et al*, 2016), immunopathological changes and neuroendocrine alterations (Tedford and McConkey, 2017). The pathogenesis of neurotoxoplasmosis was attributed to infection of neurons, microglial cells and Purkinje fibres. Bradyzoites slowly multiply inside parasitophorous vacuoles during the asymptomatic period of infection. When tissue cysts rupture, the parasite takes the tachyzoite form to either invade other cells or induce an inflammatory response by invading mononuclear cells. Activation of latent toxoplasmosis may result from various stimuli such as immunodepression or viral infection (Flegr, 2015). Toxoplasmosis neurotoxicity has been also linked to the infection-related oxidative stress generated in neuronal cells (Dincel and Atmaca, 2016). The central nervous system (CNS) is known to have a high consumption of oxygen, which makes it particularly susceptible to oxidative damage

(Andersen, 2004).

Toxoplasmosis diagnosis included bioassays, serological tests, molecular techniques, and microscopic examination of biopsied specimens. While serology was the main choice in clinical practice, molecular detection is preferable when accurate diagnosis is desired (Liu *et al,* 2015). Tailoring the diagnosis of toxoplasmosis is thus dependent on the target of investigation, whether estimation of seroprevalence or accurate diagnosis and suitable management.

The current study aimed to evaluate the role of toxoplasmosis and oxidative stress among Egyptian children suffered from neuro-developmental disorders and also, to evaluate *Toxoplasma* positive prevalence among acute infection in relation to IFN gamma with IgG & IgM.

Materials and Methods

Ethical considerations: The principle and aim of the study were explained to all parents and informed consent was obtained. The study was carried out according to the Faculty of Medicine, Cairo University Institutional Ethical Guidelines.

Study design: This study was done in Abu El-Reesh Paediatric University Hospital, Cairo University, from September 2018 to December 2018. Inclusion criteria were the acquisition of an informed consent from the mother and the age group between 2 &12 years. Clinical cases involved included patients with a variety of neurological disorders. Cross-matched control children without neurological disorders were included. Children suffered from traumatic brain injuries, CNS malignancies and those on immunotherapy were excluded from the study.

Sample and data collection: Specific diagnosis of neurological diseases was performed by a specialized pediatrician based on clinical, laboratory and radiological assessment. Information obtained from patients included age, sex and residence area. Blood samples were collected from each patient in EDTA coated plastic tubes, sera were separated and stored at -20^{o}C till use.

ELISA-IgG (Bessiéres *et al,* 2006): Anti-*Toxoplasma* IgG antibodies were detected using NovaLisa™*Toxoplasma* IgG-ELISA A Product No.TOXG0460 (Nova, TecImmundiagnostica, GmbH (D-63128 Dietzenbach, Germany).

Qualitative and quantitative measureme-nt of *T. gondii* antibodies in human serum was performed according to the manufacturer's instructions. Results were expressed as IgG International Units per milliliter (IU/ml). The resulted antibody index was considered reactive at > 35 IU/ml, equivocal at 30-35 IU/ml and non-reactive < 30 IU.

Anti-IgM antibodies (Herbrink *et al,* 1987): Anti-*Toxoplasma* IgM antibodies were detected using IgM-capture ELISA. DRG® *Toxoplasma* IgM (TORCH) (EIA-1799) (DRG International, Inc., USA) was used for detection of IgM antibodies against *T. gondii* in human sera. Results were interpreted as negative when IgM index < 0.9, equivocal when index value was 0.9 to 1.0, while positive ≥ 1.0.

Malondialdehyde (MDA) level (nmol/ mL) determination in sera (Draper and Hadley, 1990): Colorimetric measurement of serum MDA, a marker of oxidative stress, was performed using the thiobarbituric acid reaction method.

Enzyme immunoassay for avidity determination of IgG antibodies to *T. gondii* in human serum (Candolfi *et al,* 2007): IgG-ELISA NovaLisa TM *Toxoplasma gondii* IgG avidity test product number TOXGA 460 (Nova Tec Immundiagnostica GmbH (D-63128 Dietzenbach, Germany) was used for the determination of past and recent infection in IgM positive patients. Avidity was calculated according to the following equation:

Avidity % = Absorbance (sample or contrl) avidity reagent x 100.

Absorbance (sample or control) washing buffer (diluted 1 +19).

Results were interpreted as follows; avidity % > 40 indicated anti-*Toxoplasma* antibody with high avidity reflecting past infection, while avidity (%) ≤40 indicated anti-*Toxoplasma* antibody with low avidity reflecting acute or recent infection.

Molecular assay for *T. gondii* detection (Hierl *et al*, 2004): To confirm recent infec-

tion in samples which were positive for anti-*Toxoplasma* IgM, extraction of genomic DNA from collected blood samples was performed using DNA-Sorb-B kit (Sacace™ DNA-Sorb-B, Sacace Biotechnologies Srl 44 Scalabrini str., 22100 Como, Italy). The eluted DNA was tested using quantitative real time PCR (qPCR), applying SYBR® Green detection protocol & 2 primers targeting the *T. gondii* B1 gene: B1 sense (5'-GGAGG ACTGGCAACCTGGTGTCG-3') & B1 antisense (5'-TTGTTTCACCCGGACCGTTT AGCAG3').

Detection of serum INF gamma levels: Positive IgM samples from patients suffering from neurological disorders were tested for serum INF gamma levels using commercially available ELISA kit Bio (Cat#: ELH-IFN gamma-001) Ray Biotech, Inc. according to manufacturer's instructions. Assessment of INF gamma level was performed by an ELISA reader at 450nm, calculated on a standard curve and expressed in pg/mL.

Statistical analysis: Microsoft excel 2013 was used for data entry and the statistical package for social science (SPSS) version 24 (SPSS, Armonk, New York: International Business Machines Corporation) was used for data analysis. Simple descriptive statistics; arithmetic mean and standard deviation (SD), summarized quantitative data and frequencies were used for qualitative data. Chi-square test, t-independent test and one-way ANOVA were used to compare normally distributed quantitative data. The level of significance was set at probability (P) value <0.05 (Dawson and Trapp, 2004).

Results

The study included 358 children, 155 of whom were patients suffering from a variety of neurological disorders, while 203 outpatient children free of neurological diseases were taken as control. The mean age of patients was 6+/-2.5 years, while control subjects were 6.4+/-2.7. The cases consisted of 83.9% males and 16.1% females; residence was 78.8% in urban areas and 21.3% in rural areas. Neurological disorders included 25 patients (Guillain Barre syndrome; GBS), 23 (acute encephalitis), 36 (cerebral palsy), 21 (hydrocephalus), 15 (autism), 20 (Down syndrome) and 15 (attention deficit hyperkinetic disorder ADHD).

Toxoplasmosis seroprevalence and serum MDA level: Among toxoplasmosis cases 82.6% were IgG positive (titer 211.5+/-149 IU/mL) as compared to 80.8% controls (titer 186.9+/-133.6 IU/mL), but without significant difference in IgG sero-prevalence (P >0.05) or sero-intensity (P >0.05) between patients and controls. The IgM seropositive results was significantly lower as compared to controls (P <0.05), where only 10 patients were IgM seropositive (mean index value 1.4+/-0.4) as compared to 38 controls (mean index value 1.5+/-0.3). No significant difference (P > 0.05) was detected between IgM index in seropositive patients and controls**.** Evaluation of the IgG in relation to each neurological disease showed the highest percentage among patients with cerebral palsy (91.7%), and the lowest one was in children suffering from ADHD (66.7%). The IgG seropositivity in GBS, acute encephalitis, hydrocephalus, autism and Down syndrome was 80%, 73.9%, 90.5%, 73.3% & 90%, respectively. IgM seropositivity was as follows; in 4 patients suffering from acute encephalitis, 4 Down syndrome patients, and 2 cases with GBS. The MDA level in children suffering from neurological disorders was significantly higher than that found in their control counterparts. Within the cases group, the evaluation of the relation between the MDA level and sero-reactivity to anti-*Toxoplasma* IgG showed a significantly higher level in seropositive patients as compared to seronegative ones.

IgG avidity testing, qPCR, and serum IFN gamma in positive IgM patients: All IgM positive children suffered from neurological diseases as well. Avidity testing and qPCR were performed in these cases so as to investigate whether infection in these patients was an acute infection or a past one with persistent IgM. Acute infection was confirmed in

6 patients diagnosed with GBS (1 case), acute encephalitis (3 cases) and Down syndrome (2 cases). Quantitative genomic estimation of these positive samples in qPCR ranged from 2.5x 10^1 to 9x 10^8 genomic equiv lents reflecting the different DNA quantities. The IgM index in these cases (mean 1.7+/-0.4) was significantly higher than that of the four patients found to have a past infection (mean 1.2+/-0.1) by avidity and molecular testing (P <0.05). Measurement of serum IFN gamma showed significantly higher levels in confirmed acute toxop-lasmosis as compared to patients with past infection.

Details were given in tables (1, 2, 3 & 4) and figures **(1 & 2).**

Table 1: Sero-reactivity to anti-*Toxoplasma* IgG and IgM in patients and control subjects

IgG	Cases		Control		P value	IgM	Cases		Control		P value
	No.	%	No.	%			No.	%	No.	%	
Positive	128	82.6	164	80.8	> 0.05	Positive	10	6.5	38	18.7	<0.05*
Negative	27	17.4	39	19.2		Negative	145	93.5	165	81.3	
Total	155	100	203	100		Total	155	100	203	100	

*Statistically significant; Chi-square test

Table 2: MDA level in patients and control subjects

Study groups	Cases (N=155)	Control (N=203)	P-value
MDA (nmol/mL)	24.4 +/-6.8	17.5 +/-9.2	< 0.05*

*Significant; one-way ANOVA

Table 3: MDA level in patients in relation to the sero-reactivity to anti-*Toxoplasma* IgG

Study groups	IgG positive (N= 128)	IgG negative (N= 27)	P-value
MDA (nmol/mL)	24.95 +/-6.7	22.01 +/-7.1	< 0.05*

*Statistically significant; one-way ANOVA

Table 4: IFN gamma level in positive patients for IgG & IgM in relation to confirmed acute infection

Study groups	Acute infection (N= 6)	Past infection (N= 4)	P-value
IFN gamma (pg/mL)	2007.17+/-238.6	305+/-112.4	< 0.05*

*Significant; t-test

Discussion

The current study showed an overall high seroprevalence of anti-*T. gondii* antibodies in the study population. The IgG seropositivity was uniformly and widely distributed in cases (82.6%) and controls (80.8%), IgM seropositivity was significantly higher in controls (18.7%) as compared to cases (6.5%). This curious data of elevated IgM in the controls agreed with Alvarado-Esquivel *et al* (2017), but IgM was detected in 37.1% of controls and 14.3% of cases. Globally, reports estimated *T. gondii* seroprevalence to be up to 85% of the population, especially in the tropics of developing countries (Hernández-Cortazar *et al*, 2015; Nayeri *et al*, 2020). In Egypt, Abbas *et al.* (2020) stated in review on toxoplasmosis that "the current situation is confusing" without central working group conducting national level reports in the light of an apparently high *T. gondii* seroprevalence based on various sporadic studies. Ibrahim *et al* (2009) and El Deeb *et al* (2012) showed a significantly high prevalence of toxoplasmosis among pregnant females of 51.49% & 67.5%, respectively.

The present study showed various degrees of IgG seropositivity among all the neurological diseases; acute encephalitis, Down syndrome, GBS, cerebral palsy, hydrocephalus, autism and ADHD. Anti-*Toxoplasma* IgM was used as a preliminary test for acute infection. Confirmation was achieved by IgG avidity testing and qPCR for B1 *T. gondii gene*. Cases with acute toxoplasmosis included children suffering from acute encephalitis, Down syndrome and GBS.

Eraky *et al* (2016) in Benha University Hospitals found that anti-*T. gondii* IgG was significantly higher in children with cryptogenic epilepsy (20%) in comparison to children with non-cryptogenic epilepsy (0%) and healthy controls (10%). Also, Hamed *et al* (2018) showed a significantly high prevalence of anti-*T. gondii* IgG in mentally retarded children (42%) attending Cairo University hospital, when compared to the controls (17.5%). However, El-Beshbishi *et al* (2018)

in Mansoura University Hospital among children suffered from the various neurological disorders (microcephaly, hydrocephalus, ADHD & epilepsy), collectively grouped as non-syndromic neurological diseases, and Down syndrome, found that IgG seropositivity was significantly higher in non-syndromic neurologically disabled children (63.3%) and Down syndrome (13.3%), when compared to the controls (10%). Although the percentage of seropositive cases in the current study was higher in comparison to the previously mentioned ones, a significant association between presence of anti-*Toxoplasma* antibodies and neurological diseases could not be established due to the high prevalence of seropositivity also present among controls.

In general, TORCH infections (toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex virus and others such as syphilis) accounted for 5 to 10% of permanent neurological disabilities (Hermansen and Hermansen, 2006). Congenital toxoplasmosis diagnosis is easiest at birth; as serological findings later in life can be due to postnatally acquired toxoplasmosis, thus the differentiation from reactivation of congenital infection becomes challenging.

Toxoplasmic encephalitis was a serious cause of morbidity in immunocompromised individuals, seldom seen in immunocompetent patients (Habek *et al*, 2009). Though toxoplasmosis may not sound like a probable cause of Down syndrome, the possibility that it might play a role in the future development of psychiatric disease in Down syndrome patients must be considered. Dykens *et al* (2015) reported a significantly higher incidence for the development of psychotic disorders and slowing of motor and language functions in patients with Down syndrome. Reports on the causal relationship between toxoplasmosis and GBS were mainly available as individual case reports (Bossi *et al*, 1998; González *et al*, 2000; Calabuig *et al*, 2009). The importance of determining the implication of toxoplasmosis in the etiopathogenesis of GBS is highlighted by the fact that patients receiving anti-toxoplasmosis therapy shown improvement of the disease symptoms (Bossi *et al,* 1998; Calabuig *et al,* 2009).

Hydrocephalus is one of the classically described manifestation of congenital toxoplasmosis, estimated to affect about 4% of infants infected in utero. Different patterns of hydrocephalus, including those with and without anatomical obstruction were found in cases of congenital toxoplasmosis (McLeod *et al*, 2014; Hutson *et al.*, 2015). As for cerebral palsy, although congenital toxoplasmosis is a well-recog-nized risk, further updated comprehensive epidemiological studies are needed. As to ADHD, Afsharpaiman *et al* (2016) detected a seroprevalence of toxoplasmosis in 4.2% in children suffering from ADHD as compared to 2.1% of controls. But, Khademvatan *et al* (2018) showed that 18% of children and adults suffered from ADHD were toxoplasmosis seropositive as compared to 24% of controls. Though clinical evidence for linking toxoplasmosis and ADHD is lacking, experimental infection induced hyperactivity and sensorimotor changes in laboratory animals (Carter, 2013). Concerning autism, Prandota *et al* (2015) found that 23.9% of autistic patients were toxoplasmosis seropositive in contrast to 4 % in the control group. Also, the seropositive patients showed significantly higher levels of nitric oxide and IFN gamma when compared to the seronegative ones.

Being an intracellular pathogen, the immune system confronts the invading *T. gondii* by the generation of a strong Th1- cytokine response. The principal cytokine involved in the anti-*Toxoplasma* immune response is IFN gamma. In the present study, the level of IFN gamma was significantly higher in patients with acute toxoplasmosis. Melzer *et al* (2008) reported that IFN gamma lead to the attenuation of *T. gondii* growth in astrocytes through disrupting the parasitophorous vacuole thus resulting in parasite egress.

IFN gamma also compromises parasite gr-

wth by the induction of inducible Nitric oxide synthase (iNOS). The consequent generation of the cytotoxic molecule nitric oxide (NO) along with other free radicles established a state of oxidative stress (Hakimi *et al*, 2017). The brain is particularly vulnerable to the damaging effects of reactive oxygen species due to its high oxygen consumption, weak antioxidant defense mechanism and high content of polyunsaturated fatty acids. Brain pathology may result from the DNA damage, autophagy and apoptosis (Popa-Wagner *et al,* 2013).

In the current study, children with neurodevelopmental disorders had significantly higher MDA levels than their healthy counterparts. Besides, seropositive patients had statistically higher MDA levels than seronegative ones. Aycicek and Iskan (2006) reported that patients suffered from cerebral palsy were found to have higher levels of lipid peroxidation and lower antioxidant capacity levels as compared to controls. Karaman *et al.* (2008) investigated the relation between oxidative stress and toxoplasmosis by measuring the blood levels of MDA, NO and glutathione (GSH) in seropositive cases as compared with levels in seronegative ones. Significantly higher levels of MDA & NO and lower levels of GSH were associated with seropositivity to anti-*Toxoplasma* IgG. Dincel and Atmaca (2016) studied the role of oxidative stress in *Toxoplasma*-induced neuropathology and neurodegeneration in Swiss Albino mice with toxoplasmic encephalitis. They found a markedly increased in local expression of glutathione reductase & 8-hydroxy-2'-deoxyguanosine, along with decreased activity of superoxide dismutase. This oxidative state was accompanied by neuronal apoptosis and degeneration as evidenced by increased levels of neuron specific enolase.

Conclusion

The high prevalence of anti-*Toxoplasma* antibodies per se does not prove the involvement of toxoplasmosis in the etiopathogenesis of pediatric neurological diseases, even in the presence of IgM antibodies. Furthermore, MDA and IFN gamma proved important indicators of underlying infection offering potential management modalities for neurological diseased children. The overall high seroprevalence data alerts for the development of a cost-effective screening program for toxoplasmosis together with efficient plans for prevention and control.

Authors' contribution: Amin NM: Setting of study concept; Designed study plan, material supplementation carried out laboratory analysis and data recording. El-kazaz A: Data and sample collection and patient evaluation. Abdel-Shafi IR: Review & editing. El-Sebaei EH: Data analysis and statistics. Abdeltawab MSA: Interpretation of data; writing of original draft; review & editing. All authors critically revised the manuscript and approved the final manuscript.

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References

Abbas, IE, Villena, I, Dubey, JP, 2020: A review on toxoplasmosis in humans and animals from Egypt. Parasitology 147, 2:135-59.

Afsharpaiman, S, Khosravi, MH, Faridche hr, M, *et al*, 2016: Assessment of *Toxoplasma* seropositivity in children suffering from attention deficit hyperactivity disorder. Galen Med. J. 5, 4:188-93.

Alharbi, EH, Yamani, LK, Taiyeb, AE, *et al*, 2017: Seroprevalence and potential root cause of Toxoplasmosis in primary children; a study conducted at King Abdulaziz Hospital. Egypt. J. Hosp. Med. 66, 1:188-92.

Alvardo-Esquivel, C, Rico-Almochantal, YD R, Hernandez-Tinoco, J, *et al*, 2017: *Toxoplasma gondii* exposure and neurological disorders: an age and gender matched case-control pilot study. Eur. J. Microbiol. Immunol. 7, 4:303-9.

Andersen, J, 2004: Oxidative stress in neurodegeneration: cause or consequence? Nat. Med. 10: S18-25.

Aycicek, A, Iscan, A, 2006: Oxidative and antioxidative capacity in children with cerebral palsy. Brain Res. Bull. 69, 6:666-8.

Bassiony, H, Soliman, NK, El-Tawab, S, *et al*,

2016: Sero-prevalence and risk factors associated with *Toxoplasma gondii* infection among pregnant women in Alexandria, Egypt. Int. J. Reprod. Contracept. Obstet. Gynecol. 5, 12: 4220-7.

Bessiéres, MH, Roques, C, Berrebi, A, *et al*, 2006: Toxoplasmosis: epidemiology, clinical manifestation and infection in pregnant women. Przegl. Lek. 63, 2:97-9.

Bossi, P, Caumes, E, Paris, L, *et al*, 1998: *Toxoplasma gondii*-associated Guillain-Barré syndrome in an immunocompetent patient. J. Clin. Microbiol. 36, 12:3724-5.

Calabuig, E, Roig, P, Castaño, D, *et al*, 2009: P0714 *Toxoplasma gondii* associated to Guillain Barre Syndrome in inmunocompetent patient. Eur. J. Intern. Med. 20, 1:S233.

Candolfi, E, Pastor, R, Huber, R, *et al,* 2007: IgG avidity assay firms up the diagnosis of acute toxoplasmosis on the first serum sample in immunocompetent pregnant women. Diagn. Microbiol. Infect. Dis. 58, 1:83-8.

Carter, CJ, 2013: Toxoplasmosis and polygenic disease susceptibility genes: Extensive *Toxoplasma gondii* host/pathogen interactome enrichment in nine psychiatric or neurological disorders. J. Pathog. 2013:e965046.

David, CN, Frias, ES, Szu, JI, *et al*, 2016: GLT-1-dependent disruption of CNS glutamate homeostasis and neuronal function by the protozoan parasite *Toxoplasma gondii.* PLoS Pathog. 12, 6:e1005643.

Dawson, B, Trapp, R, 2004: Basic and clinical biostatistics, 4th Ed., New York, United States, Mcgraw-Hill Medical Publishing Inc.

Dincel, GC, Atmaca, HT, 2016: Role of oxidative stress in the pathophysiology of *Toxoplasma gondii* infection. Int. J. Immunopathol. Pharmacol. 29, 2:226-40.

Draper, HH, Hadley, M, 1990: Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 186:421-31.

Dykens, EM, Shah, B, Davis, B, *et al*, 2015: Psychiatric disorders in adolescents and young adults with Down syndrome and other intellectual disabilities. J. Neurodev. Disord. 7, 1:9-12.

El-Beshbishi, SN, El-Tantawy, NL, Elzeky, S M, *et al*, 2018: Seroprevalence of *Toxoplasma gondii* infection in children with central nervous system disorders in Mansoura, Egypt: A case-control study. Trans. R. Soc. Trop. Med. Hyg. 112, 12:555-60.

El Deeb, HK, Salah-Eldin, H, Khodeer, S, *et al*, 2012: Prevalence of *Toxoplasma gondii* infection in antenatal population in Menoufia governorate, Egypt. Acta Trop. 124, 3:185-91.

Eraky, MA, Abdel-Hady, S, Abdallah, K F, 2016: Seropositivity of *Toxoplasma gondii* and *Toxocara* spp. in children with cryptogenic epilepsy, Benha, Egypt. Korean J. Parasitol. 54, 3: 335-8.

Flegr, J, 2013: Influence of latent *Toxoplasma* infection on human personality, physiology and morphology: pros and cons of the *Toxoplasma*-human model in studying the manipulation hypothesis. J. Exp. Biol. 216:127-33.

Flegr, J, Pradonta, J, Sovickova, M, *et al*, 2014: Toxoplasmosis, a global threat: Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. PLoS One 9, 3: e90203.

Flegr, J, 2015: Neurological and neuropsychiatric consequences of chronic *Toxoplasma* infection. Curr. Clin. Micro. Rpt. 2:163-72.

Gavrilesu, LC, Denkers, EY, 2001: IFN-γ overproduction and high-level apoptosis are associated with high but not low virulence *Toxoplasma gondii* infection. J. Immunol. 167, 2:902-9.

González, MI, Caballero, D, Lopez C, *et al*, 2000: Cerebral toxoplasmosis and Guillain-Barré syndrome after allogeneic peripheral stem cell transplantation. Transpl. Infect. Dis. 2:145-9.

Habek, M, Ozretic, D, Zarkovic, K, *et al*, 2009: Unusual cause of dementia in an immunocompetent host: toxoplasmic encephalitis. Neurol. Sci. 30, 1:45-9.

Hakimi, MA, Olias, P, Sibley, LD, 2017: *Toxoplasma* effectors targeting host signaling and transcription. Clin. Microbiol. Rev. 30:615-45.

Hamed, AMR, El-Gebaly, NSM, Abdelmegeid, AK, *et al*, 2018: Seroprevalence of *Toxoplasma gondii* infection in mentally retarded children in Egypt. PUJ 11, 3:155-61.

Herbrink, P, Van-Loon, AM, Rotmans, JP, *et al*, 1987: Interlaboratory evaluation of indirect enzyme-linked immunosorbent assay, antibody capture enzyme-linked immunosor-bent assay and immunoblotting for detection of immunoglobulin M antibodies to *Toxop-lasma gondii*. J. Clin. Microbiol. 25:100-5.

Hermansen, MC, Hermansen, MG, 2006: Perinatal infections and cerebral palsy. Clin. Perinatol. 33, 2:315-33.

Hernández-Cortazar, I, Acosta-Viana, KY,

Ortega-Pacheco, A, *et al*, 2015: Toxoplasmosis in Mexico: epidemiological situation in humans and animals. Rev. Inst. Med. Trop. SP. 57, 2:93-103.
Hierl, T, Reischl, U, Lang, P, *et al,* 2004: Preliminary evaluation of one conventional nested and two real-time PCR assays for the detection of *Toxoplasma gondii* in immunocompromised patients. J. Med. Microbiol. 53:629-32.
Hill, D, Dubey, JP, 2002: *Toxoplasma gondii*: Transmission, diagnosis, and prevention. Clin. Microbiol. Infect. 8, 10:634-40.
Hutson, SL, Wheeler, KM, McLone, D, *et al*, 2015: Patterns of hydrocephalus caused by congenital *Toxoplasma gondii* infection associate with parasite genetics. Clin. Infect. Dis. 61, 12: 1831-4.
Ibrahim, HM, Huang, P, Salem, TA, *et al,* 2009: Short report: prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in northern Egypt. Am. J. Trop. Med. Hyg. 80, 2:263-7.
Karaman, Ü, Çelik, T, Kıran, TR, *et al*, 2008: Malondialdehyde, glutathione, and nitric oxide levels in *Toxoplasma gondii* sero-positive patients. Korean J. Parasitol. 46, 4:293-5.
Khademvatan, S, Riahi, F, Izadi-Mazidi, M, *et al*, 2018: *Toxoplasma gondii* exposure and the risk of attention deficit hyperactivity disorder in children and adolescents. Pediatr. Infect. Dis. J. 37, 11:1097-100.
Liu, Q, Wang, ZD, Huang, SY, *et al*, 2015: Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. Parasit. Vectors 8:292-4.
McLeod, R, Lykins, J, Gwendolyn Noble, A, *et al,* 2014: Management of congenital toxoplasmosis. Curr. Pediatr. Rep. 2:166-94.
Melzer, T, Duffy, A, Weiss, LM, *et al*, 2008: The gamma interferon (IFN-gamma)-inducible GTP-binding protein IGTP is necessary for *Toxoplasma* vacuolar disruption and induces parasite egression in IFN-gamma-stimulated astrocytes. Infect. Immun. 76, 11:4883-94.
Nayeri, T, Sarvi, S, Moosazadeh, M, *et al*, 2020: The global seroprevalence of anti-*Toxoplasma gondii* antibodies in women who had spontaneous abortion: A systematic review and meta-analysis. PLoS Negl. Trop. Dis. 14, 3:e0008103.
Popa-Wagner, A, Mitran, S, Sivanesan, S, *et al*, 2013: ROS and Brain Diseases: The Good, the Bad, and the Ugly; Oxid. Med. Cell. Longev. Volume 2013: 963520.
Prandota, J, Elleboudy, NA, Ismail, KA, *et al*, 2015: Increased seroprevalence of chronic toxoplasmosis in autistic children: special reference to the pathophysiology of IFN-γ and no overproduction. Int. J. Neurol. Res. 1, 3:102-22.
Remington, JS, McLeod, R, Wilson, CB, *et al*, 2017: Toxoplasmosis. In: Infectious Diseases of the Fetus and Newborn Infant, 7th ed, Remington, JS, Klein, JO, Wilson, CB, *et al* (Eds), Elsevier Saunders, Philadelphia.
Tedford, E, McConkey, G, 2017: Neurophysiological changes induced by chronic *Toxoplasma gondii* infection; Pathogens 6, 2:19-22.

Explanation of figures

Fig.1: Pie chart representing the percentage of each neurological disease relative to cases (N=155). GBS= Guillain Barre syndrome, AE= acute encephalitis, CP= cerebral palsy, HP= hydrocephalus, DS= Down syndrome, ADHD= attention deficit hyperkinetic disorder

Fig. 2: Column chart representing the sero-reactivity to anti-*Toxoplasma* IgG in relation to each neurological disease. GBS= Guillain