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THE RELATIONSHIP BETWEEN TOXOPLASMA GONDII AND AUTISM SPECTRUM DISORDERS

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Abstract

The current study was conducted at in autism center during the period from June 2022 to February 2023. nine blood samples were collected randomly autism center in Al Najaf province. All samples were screened for T. gondii IgG and IgM antibodies using immunochromatographic test (immunochromatographic, On Site Toxo IgG/IgM Combo Rapid Test) to confirm T. gondii infection. immunochromatographic test was performed blood, which were collected from sample for immunochromatographic. The clinical assessment of the 11 patients (who confirmed to have toxoplasmosis a using ICT) was revealed that the frequency of distribution of patients according to sex were 1 (2%) female and 10 (98%) males. The differences between males and females were statistically significant ($P < 0.05$, P-value= 0.001).

Keywords: Toxoplasmosis, Toxoplasma gondii, protozoan, immunochromatographic test, autism.

Introduction

Toxoplasmosis is a parasitic disease caused by the intracellular protozoan Toxoplasma gondii, which affects all warm-blooded animals, including humans. Toxoplasmosis is one of the most common parasitic diseases that causes reproductive loss in animals (Ferra et al., 2020). Toxoplasmosis is a neglected tropical disease with a global distribution that is estimated to infect one third of the world's human population (Onosakponome et al., 2020).

Infection may occur from oocyst-contaminated food, water, or raw meat products containing parasite cysts, as well as infection from the mother to the fetus. The parasite's life cycle is complete as it passes from warm-blooded intermediate hosts to the cat as the final host (Djurkovic-Djakovic et al., 2019).

Infections are normally asymptomatic or develop moderate symptoms that are self-limiting, but infections in immunocompromised people may be severe. Infections in pregnant women can result in severe health issues in their children, including mental retardation and blindness. In immunocompetent adults, T. gondii infection may cause vision loss, Toxoplasmosis has ranked very greatly in two studies of death and incapacity attributable to foodborne pathogens. The ingestion of raw or undercooked meat containing Toxoplasma gondii tissue cysts and the consumption of raw vegetables or water contaminated with T. gondii oocysts from cat feces is most commonly Linked with human illness. The risk of acquiring a Toxoplasma infection through food varies with cultural and eating habits in diverse human populations (Hussain et al., 2017).

Autism, heterogeneous neurodevelopmental disorder, is a behaviorally defined condition that is typically diagnosed after the second year of life, and about 1 in 68 children is affected by the disease (Lim et al., 2013 and Ling et la., 2011). According to the Diagnostic and Statistical Manual of Mental Disorder (DSM-V) criteria, the symptoms of ASD are persistent deficits in social communication interactions and lead to restricted-repetitive patterns of behavior, interests, or activities (Ling et al , 2011; Lim et al ., 2013) As mentioned above and also given the contradictory results of few studies that examined the relationship between autism and Toxoplasma, the main objective of the current study was to summarize the evidence and to estimate the total OR for the association between T. gondii infection and ASD.

The aim of this study is The link between people with autism spectrum disorders and with Toxoplasmosis.

1. Toxoplasmosis

Toxoplasmosis is caused by infection with Toxoplasma gondii. This coccidian protozoan is normally transmitted to humans by ingestion of either oocysts from cat feces, or by tissue cysts in raw or undercooked meat. It is one of the most important problems in immunocompromised individuals. In immunocompetent individuals, toxoplasmosis is generally asymptomatic. Lymphadenopathy is the most common manifestation in the 10% - 20% of immunocompetent individuals whose primary T. gondii infection is symptomatic. Chorioretinitis, myocarditis and/or polymyositis are less frequently presented in these patients, but can occur (Montoya and Remington,

2000; Montoya, 2002). During pregnancy, an infection with Toxoplasma can result in either fetal death or in a child with physiological, physical or neurological defefects. Fetal contamination is estimated to occur in 0.1 to 0.5% of infected pregnant women in the United Kingdom, 0.2 to 0.3% in Southern Finland, 1% in France, 0.2 to 0.8% in Canada, and 0.2 to 0.6% in the United States (Nguyen et al., 1996).

2. History:

Toxoplasma gondii was first discovered in an African desert rodent, Ctenodactylus gundi, in 1908 by Nicolle and manceaux (Dubey and Beattie, 1988) and described the same year by Samuel T. Darling (De la Cruz, 1989). The name was derived from the Greek toxon, bow or arc, alluding to lunate shape (Frenkel, 1973). It was then found in many species of mammals and birds worldwide and also in humans. In 1923, Janku described T. gondii in the retina of a hydrocephalic child, but the role of the parasite as a human pathogen was not widely known until Wolf and Cowen reported congenital T. gondii infection in man. Their report stimulated considerable interest in human toxoplasmosis. Pinkerton and Weinman reported the first known cases of fatal toxoplasmosis in adult human patients. The development of the dye test by Sabin and Feldman was and still is the key to much of our present knowledge of toxoplasmosis (Dubey and Beattie, 1988). In 1970, the life cycle of T. gondii was first described. Until then Isospora species were considered parasites of carnivores (dogs, cats) and birds and were thought not to be host specific. T. gondii, was first known to parasitize extraintestinal tissues of virtually all warm-blooded hosts, but then was found to be an intestinal coccidia of cats and to have in its life cycle an isosporan-like oocyst. Further life cycle studies indicated that some of the Isospora species that had been considered to have only an intestinal cycle also had stages in extraintestinal tissues (Dubey, 1993).The knowledge of T. gondii life cycle was completed by the finding of the sexual phase of the parasite in the small intestine of the cat. Toxoplasma gondii oocysts, the product of the merogony and gametogony, were found in cat feces and characterized morphologically and biologically (Dubey et al., 1970a). In 1977, an outbreak of toxoplasmosis involved patrons of a riding stable in Atlanta (Teutsch et al., 1979). Unlike other reported outbreaks that have been caused by eating raw or undercooked meat, this outbreak was caused by oocysts from cat feces (Dubey et al., 1981). An unusual high rate of clinical diseases was associated with this outbreak. Thirty five (95%) of the patrons of the stable who had laboratory evidence of infection had clinical illness characterized by fever, lymphadenopathy, and headache (Dubey et al., 1981). In 1984, an epidemic of toxoplasmic encephalitis occurred in patients with AIDS (Luft et al., 1984; Wanke et al., 1987). In 1995, the first large outbreak of

toxoplasmosis to be associated with municipal drinking water occurred in British Columbia, Canada. This was one of the largest outbreaks reported until that date. One hundred and ten acute Toxoplasma infections were identified. Of these, 42 infected women and 11 newborns were identified as well as 57 infections in non-pregnant individuals (Bowie et al., 1997; Bell et al., 1995). In 2001, the largest outbreak of toxoplasmosis ever reported in the world occurred in Parana, Brazil, affecting 290 people, of which 176 became ill, including three pregnant women. The vehicle was again contaminated water (Promed, 2002).

3. Classification and Toxoplasma gondii strains

The life cycle of Toxoplasma gondii was proposed more 25 years ago. Since that time, despite attempts to make the genus polyspecific, there has been only one species, Toxoplasma gondii, consistently recognized in the genus. Recent studies have been done to investigate the genetic diversity among strains in the species T. gondii. Results of this analyses confirm that the strain in the genus Toxoplasma comprise a limited number of clonal lineages, directly correlated with their virulence in mice (Johnson, 1998). The genus Toxoplasma is classified in the Phylum Apicomplexa (Levine, 1977), class Sporozoasida (Leukart, 1879), subclass Coccidiasina (Leukart, 1879), order Eimeriorina (Leger, 1911), family Toxoplasmatidae (Dubey, 1993). Only one species of the genus Toxoplasma has been observed to exist so far (Guo and Johnson, 1996), however, there are large differences in virulence among the various strains and isolates of T. gondii. This virulence diversity among isolates may have considerable impact on epidemiology, immunology, pathology and the parasite-host relationship. Some strains such as the extremely virulent RH strain have lost the ability to form oocysts in the cat, and as few as ten tachyzoites can be lethal for a mouse within a week when injected intraperitoneal (Guo and Johnson, 1996). The RH strain was isolated in 1939 from the brain of a 6 year-old boy. Other strains such as the avirulent S-1 strain form cysts in the brains of mice injected with 1000 oocysts (equivalent to 8000 tachyzoites) and these mice survive without ill effects (Dubey and Frenkel, 1973).

In human toxoplasmosis, the role of the infecting Toxoplasma strain is not easily assessed although it is clear that infections can have dramatically different outcomes. There is overwhelming evidence that the immunological status of the host plays a major part in the clinical presentation of this opportunistic infection (Darde, 1996).

Kingdom Protesta
Phylum Apicomplexa
Class Sporozoa
Subclass Coccidiasina

Order Eucoccidiorida
Suborder Eimeriorina
Family Sarcocystidae
Subfamily Toxoplasmatinae
Genus Toxoplasma
Species gondii.

4. Morphology and Structure

Scanning and transmission electron microscopy have been used to obtain a large number of images of the various developmental stages of T. gondii. The three infective forms (i.e. tachyzoite, bradyzoite and sporozoite) present the same primary organization, displaying an elongated shape and a typical apical complex where structures and organelles such as the conoid, micronemes, and rhoptries are found (De Souza and Attias, 2015).

5. Life cycle of Toxoplasma gondii

Toxoplasma gondii is a parasite whose life cycle classically includes transmission from definitive hosts (felids), in which sexual reproduction occurs, to intermediate hosts (warm-blooded vertebrates), in which it multiplies asexually(Figure 1) (Dubey 2014). There are various routes that can lead to infection in human beings, directly or indirectly, with the contamination of T. gondii in food and the environment. Humans can be infected by the ingestion of oocysts from contaminated water, soil, vegetables and fruits, intake of undercooked or uncooked meat containing tissue cysts, unpasteurized goat's milk and by the congenital transmission of tachyzoites from a non-immune mother to her foetus (VanWormer et al., 2013;Dubey et al.,2020). Making this zoonotic parasite one of the most widespread human pathogens in the world. Infected felids excrete up to several hundred million environmentally resistant oocysts with their feces, which can infect any warm-blooded animal upon ingestion. There, T. gondii reproduces asexually via two distinct life cycle stages, the fast growing tachyzoite and the slower reproducing bradyzoite stage. The latter forms cysts in various host tissues, which may be consumed by carnivores or omnivores. Following ingestion, bradyzoites are released from cysts, reverting to the tachyzoite stage, replicating, and invading surrounding tissues before eventually disseminating throughout the body to other organs (Blader et al., 2015).

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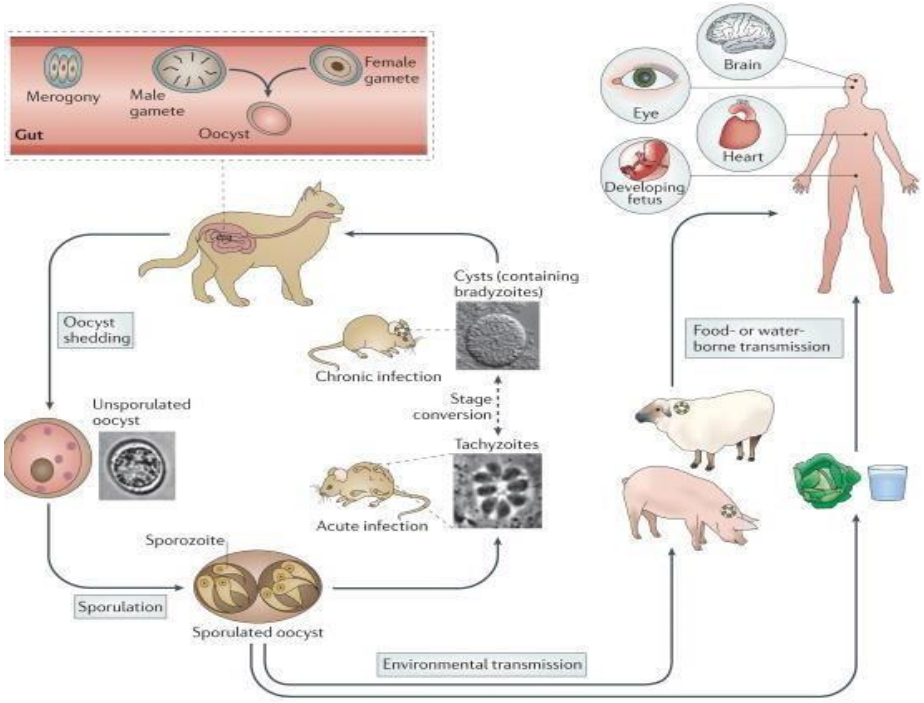

Figure 1. The complex life cycle of Toxoplasma gondii (Hunter and Sibley, 2012)

6. Pathogenesis

Toxoplasmosis, a zoonotic disease, is caused by obligate intracellular parasitic protozoa, T. gondii, leading to various clinical symptoms including encephalitis, chorioretinitis, mental retardation, loss of vision in congenitallyinfected children and abortion in livestock (Tilahun et al.,2018).

6.1 Ocular toxoplasmosis

Ocular toxoplasmosis (OT) is caused by the parasite Toxoplasma gondii and affects many individuals throughout the world. Infection may occur through congenital or acquired routes. The parasites enter the blood circulation and reach both the retina and the retinal pigment epithelium, where they may cause cell damage and cell death (Rodriguez Fernandez et al.,2021).

6.2 Congenital toxoplasmosis

Congenital toxoplasmosis is one of the most important forms of infection among the diseases that affect fetuses. The infection is caused by Toxoplasma gondii and can result in fetal infeasibility or clinical manifestations at any stage of the child's life. In addition, there is an estimated global incidence of 1.5 cases per 1000 live births (Torgerson and Mastroiacovo 2013).

6.3 Epidemiology:

The frequency of primary maternal toxoplasma infection depends on the proportion of seronegative pregnant women who are susceptible to infection and on the prevailing infection risk (Stray-Pedersen et al., 1993). After primary infection, lifelong immunity develops, which can be demonstrated by the presence of toxoplasma immunoglobulin G (IgG). The prevalence of toxoplasma IgG in women of fertile age indicates indirectly the general susceptibility to infection in the pregnant population as a whole. Knowledge of the prevalence is important because preventive guidelines are most often based on this information. The parasite T. gondii exists worldwide with considerable variations between different geographical areas and between different populations within one area (Ades et al., 1993). The worldwide prevalence of toxoplasma IgG antibody among the pregnant population or among females of reproductive age ranges from 1 to 84%. The highest prevalence has been reported in Africa, South East Asia and Central America with the lowest in Japan, Korea and the northern parts of Scandinavia (Pappas et al., 2009). Autism, heterogeneous neurodevelopmental disorder, is a behaviorally defined condition that is typically diagnosed after the second year of life ,and about 1 in 68 children is affected by the disease [12,13]. According to the Diagnostic and Statistical Manual of Mental Disorder (DSM-V) criteria, the symptoms of ASD are persistent deficits in social communication interactions and lead to restricted-repetitive patterns of behavior, interests, or activities (Ling et al , 2011; Lim et al ., 2013) .As mentioned above and also given the contradictory results of few studies that examined the relationship between autism and Toxoplasma, the main objective of the current study was to summarize the evidence and to estimate the total OR for the association between T. gondii infection and ASD.

6.7 Diagnosis

The combination of serology and molecular methods has been used to improve the diagnosis of infection by T. gondii. One of them reported that IgG Anti-T. gondii antibody low avidity correlates positive PCR in pregnant women (Olariu et al., 2019).

6.8 Direct Microscopically Examination

The detection of T. gondii oocysts in fecal, water, environmental, and tissue samples has traditionally relied on microscope examination. However, identification based on light microscopy alone is less sensitive and unreliable. The oocysts form of the parasite in fecal samples, water resources, and environment can be enriched from large volumes of samples by filtration or centrifugation for examination, and the tissue cysts

can be stained, which helps to distinguish the parasites from host cells. Giemsa and Haematoxylin and Eosin (HE) staining are simple, cost-effective, and commonly used for this purpose (Kotresha and Noordin 2010).

6.9 Serological Tests

Several serological events are available for the detection of T. gondii antibody in patients, which may assist diagnosis, Antibody detection is usually used for the diagnosis of toxoplasmosis. High frequency of IgG antibodies is an indication that the person is previously infected. Detection of specific IgM antibodies show a recent infection. Human toxoplasmosis can be diagnosed typically using Indirect Fluorescent Antibody Testing (IFAT), Latex Agglutination Test (LAT), Immuno-chromatographic Test (ICT), or Enzyme Linked Immunosorbent Assay (ELISA) (Gyang et al., 2015).

6.9.1 Mmunochromatography test (ICT)

ICT is a rapid lateral flow test intended to detect the presence or absence of the target analyte (Wang et al.,2011). This is a low-cost qualitative test detecting simultaneously specific anti Toxoplasma IgG and IgM antibodies. According to several studies, Toxoplasma ICT IgG-IgM has a high sensitivity for IgG detection, Furthermore, this ICT test was able to distinguish between nonspecific IgM and specific IgM (Rym et al., 2021). Its ease of application and rapidity of test results with no special equipment required makes the ICT suitable for field application. In toxoplasmosis, this technology has been used to diagnose human (Wassef and Abdel-Malek 2019). ICT has been shown to have a high agreement with ELISA in terms of sensitivity and specificity (Ybañez and Nishikawa 2020).

6.9.2 Latex Agglutination Test (LAT)

Agglutination tests require particulate antigens that can bind with antibodies. Multivalent antibodies (called agglutinins) form large clumps or aggregates with suspended particulate antigens when present, which can be visually seen without magnification. These tests are used to determine concentrations of specific antibodies. In toxoplasmosis diagnosis in humans and animals, different agglutination tests (Dubey, 1997, 2008; Robert-Gangneux and Dardé, 2012; Liu et al., 2015). 1.10.2.3 Indirect Fluorescent Assay (IFA) IFA is an alternative simple and safe diagnostic method that does not use live tachyzoites (Rorman et al., 2006; Saraei et al., 2010). This assay is based on the specific antigen-antibody interaction from diluted serum specimens with killed Toxoplasma tachyzoites. The interaction will then be detected by the addition of fluorescent-labeled anti-human IgG or IgM antibodies under a

fluorescence microscope (Pappas et al., 1986). Among the limitations of IFA include the individual differences in result reading and the chances of false-positive results in case the sera contain rheumatoid factors or antinuclear antibodies (Rorman et al., 2006).

6.9.3 Enzyme Linked Immuno sorbent Assay (ELISA)

ELISA is still considered one of the most common techniques with high sensitivity and specificity in the quantitative detection of antibodies and all antigenically active molecules, The ELISA system typically consists of a solid phase antigen or antibody, enzyme-labeled antigen or antibody, and a substrate for the enzyme reaction, which can be modified to test both antibodies and antigens (Liu et al.,2015). IgM antibodies are present in the acute phase of the infection and reach high levels within a month; however, due to the half-life of antibodies, these become undetectable after a few week (Murat et al., 2015). In some cases, anti-T. gondii IgM can be detected for more than a year, which makes diagnosis difficult, especially in cases with suspected infection in the first trimester of pregnancy (Liu et al., 2015).

Conclusion

The study aimed to investigate the association between Toxoplasma gondii infection and autism spectrum disorders (ASD). Toxoplasmosis, caused by T. gondii, is a common parasitic disease affecting both humans and animals globally. It is transmitted through various routes, including ingestion of oocysts from contaminated food or water, or by consuming raw or undercooked meat containing tissue cysts. The parasite's life cycle involves both warm-blooded intermediate hosts and cats as the final host. Infection in humans is usually asymptomatic or causes mild symptoms, but can lead to severe complications in immunocompromised individuals and pregnant women, potentially affecting the fetus. The ASD is a neurodevelopmental disorder characterized by deficits in social communication and repetitive behaviors. The study aimed to explore the relationship between T. gondii infection and ASD, given the conflicting results from previous studies. The researchers conducted clinical assessments and serological tests on a group of patients with ASD to detect T. gondii antibodies. They found a significant association between T. gondii infection and ASD, with a higher prevalence of infection among male patients. The findings suggest a potential link between T. gondii infection and ASD, highlighting the importance of further research to understand the underlying mechanisms. Early detection and treatment of T. gondii infection could potentially help in managing ASD symptoms.

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