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Review

Relationship between toxoplasmosis and autism: A systematic review and meta-analysis

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ABSTRACT

Toxoplasma gondii (*T. gondii*) is a foodborne parasite that is investigated in many psychiatric diseases, such as autism spectrum disorders (ASD). Therefore, a systematic literature review was conducted searching seven electronic databases on the prevalence of *T. gondii* antibodies among autism patients. The current study involved sensitivity analysis, meta-regression, subgroup analysis, publication bias test, and quality assessment of studies. On the basis of the findings, the odds ratio (OR) of latent *Toxoplasma* infection 1.93 (95% confidence intervals (CI): 1.01–3.66) was associated with ASD risk. However, there was no relationship between acute infection and ASD 0.39 (95% CI: 0.18–0.87). The obtained results of Begg's and Egger's tests showed no publication bias ($P = 0.851$ and $P = 0.297$, respectively). The sensitivity analysis confirmed robust and stable estimates with a significant level of heterogeneity ($I^2 = 78.1\%$, $P < 0.000$). Of the investigated patients' characteristics, only the gender variable was analyzed, indicating the combined ORs of 2.63 (95% CI: 0.29–23.63) in females and 2.62 (95% CI: 0.94–7.30) in male participants. This study showed that toxoplasmosis plays an important role as a risk factor for autism. However, further prospective investigations are highly recommended to illuminate the developmental pathways to this disorder and provide new strategies for the prevention and treatment of this disease.

1. Introduction

About one-third of inhabitants on Earth are infected with the coccidian parasite *Toxoplasma gondii* (*T. gondii*) [1,2].Geographic location, lifestyle, cultural traditions, and personal health habits are among the factors that affect the survival and distribution of *T. gondii* [3]. *T. gondii* infection has two phases of acute and chronic in intermediate hosts. The acute infections promoted by rapidly dividing tachyzoites are generally subclinical and asymptomatic in healthy individuals. The dormant stage of toxoplasmosis is defined as the presence of slowly dividing bradyzoites of *Toxoplasma* in many tissues, such as brain cells. As a result, mortality in people with immunodeficiency and abortion in pregnant women are suspected as the consequences of *T. gondii* infection [2,4]. It was reported that *T. gondii* may cause damage to certain areas of the brain in childhood autism. *T. gondii* can increase dopamine levels, which may affect human behavior and cause psychiatric illnesses [5]. The *T. gondii* interacts with 3000 host genes during its life cycle, which may include genes related to psychiatric disorders (e.g., obsessive-compulsive disorder, depression, epilepsy, and autism spectrum disorders [ASD]) as well as autoimmune disorders (e.g., multiple sclerosis and rheumatoid arthritis) [5]. Recently, several systematic review and meta-analysis studies have examined the relationship between *T. gondii* infection and various mental and autoimmune diseases, such as obsessive-compulsive disorder [6], Parkinson's disease [7], epilepsy [8], depression [9], rheumatoid arthritis [10] and multiple sclerosis [11]. The results of these studies showed that *T. gondii* could be a risk factor for obsessive-compulsive disorder, epilepsy, and rheumatoid arthritis, but there is no relationship between *Toxoplasma* and depression, Parkinson's disease, and multiple sclerosis.

Autism, heterogeneous neurodevelopmental disorder, is a

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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 [14,15].

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.

2. Methods

2.1. Design and protocol registration

The current study was conducted based on the PRISMA protocol for systematic reviews and meta-analysis (Supplementary 1) [16]. The study protocol has been registered in the PROSPERO, with the registration number of CRD42019124754 [17].

2.2. Search strategy

In order to evaluate the association between *T. gondii* infection and ASD, a systematic search of relevant papers in seven electronic databases (PubMed, ScienceDirect, Scopus, ProQuest, Web of Science (ISI), EMBASE, and Google Scholar) was conducted up to April 17, 2019 regardless of publication date and language (Supplementary 2). The search process was accomplished using the following keywords alone or in combination: "toxoplasmosis" OR "*Toxoplasma gondii*" OR "*T. gondii*" AND "autism" OR "autism spectrum disorder" OR "ASD".

2.3. Study selection

All the retrieved articles were saved in EndNote (version X9) to organize the summaries. Afterward, "Find Duplicates" command in EndNote software was used to select the auto-searched duplicates. The duplication search included a comparison of the authors' name, journals' name, volume number, and the titles. In the next step, two investigators read the titles and abstracts independently. Subsequently, the full texts of eligible articles were reviewed and texts with inclusion criteria were selected for the current systematic review. In case of discrepancies, the researchers discussed the issue with a third reviewer and tried to reach a consensus on the inclusion or exclusion of the article. Finally, there was no disagreement between the two reviewers and the coefficient of agreement (kappa) was 100%.

2.4. Inclusion and exclusion criteria

Studies meeting the following criteria were considered eligible 1) case-control and cross-sectional studies investigating the relationship between toxoplasmosis and ASD, 2) the studies where toxoplasmosis was diagnosed by detecting antibodies (IgG, IgM, and IgA) against *T. gondii* in subjects with a definitive diagnosis of ASD according to the DSM-V criteria, 3) studies whose results are reported as odds ratio (OR) with 95% confidence interval (CI) or can be calculated, and 4) original articles with available full text in all languages. Non-human studies, dissertations, conference papers, reviews, and protocol articles were excluded from the systematic review.

2.5. Data extraction

TN and ZH extracted the data related to the retrieved papers from seven electronic databases. In the next step, two authors simultaneously performed data extraction from each eligible study into a Microsoft Excel datasheet for the following items: first author's last name, year of publication, place of study, type of study, diagnostic method for *Toxoplasma* infection, type of antibody, mean age, sex, number of total participants, number of cases and controls, number of infected subjects in each group, OR, and P-value. The lists of extracted information are presented in Table 1.

2.6. Quality assessment

The quality of the included reports was compared using the Newcastle-Ottawa Scale (NOS) for non-randomized studies, including case-control and cohort studies [18]. This checklist includes eight items for case-control and six items for cross-sectional studies in three different categories, namely selection, comparability, and exposure. One of the items contains two options that can be scored as two.

2.7. Statistical analysis

The obtained data were analyzed using Stata software (version 14; Stata Corp, College Station, TX, USA) [25]. The heterogeneity among the studies was tested by I^2 statistic. Moreover, random effect models were used for all meta-analyses to calculate pooled ORs and 95% CIs. First, for each study, a two-by-two table containing the numbers individuals with autism spectrum disorders, individuals without autism spectrum disorders, individuals with autism spectrum disorders and *Toxoplasma* positive, and individuals without autism spectrum disorders and *Toxoplasma* positive was extracted. Then, using the metan command, the OR and 95% CI were calculated for each study and overall estimate. For I^2 values greater than 75%, the strong heterogeneity suggested the performance of an additional meta-regression. In order to provide a comprehensive analysis of the studies included in the meta-analysis, a Forest Plot chart was designed according to OR and CI. Furthermore, the current study benefited from sensitivity and subgroup analyses of articles. The publication bias was examined using Begg's and Egger's tests.

3. Results

The search process resulted in the identification of 4195 studies (Fig. 1). After the exclusion of duplicates, 4058studies remained for further analysis. The screening of the titles and abstracts also led to the removal of 4016 articles. Therefore, 42 articles were assessed for eligibility through a full-text review. After the exclusion of 32 review studies and non-relevant articles, 10 articles were included in the systematic review [1,2,5,13,19–24]. Out of 10 studies, a total of 4 papers were excluded due to the lack of control group [19], the examination of immunoglobulin levels in autism patients [13,24], and the use of a control group with abnormalities other than autism [22]. With regard to the language of publication, nine studies were published in English [1,2,5,13,19,21–24], and only one study was in Persian [20] during 2010–2017.

A total of 132 patients with autism and 24336 controls in cross-sectional studies and 1062 patients with autism and 1015 controls in case-control studies were included in the meta-analysis. The investigated articles were from different countries, such as Czech Republic [1,2], Egypt [21,22], United States [13,24], Turkey [5], Iran [20], Greece [19], and Finland [23] (Table 1). Regarding the subject of investigation, 1, 7, and 8 studies had addressed IgA antibody [13], anti-*T. gondii* IgM [2,5,13,20,22–24], and anti-*T. gondii* IgG [2,5,13,20–24], respectively, using different diagnostic methods, such as enzyme immunoassays (EIA), enzyme-linked immunosorbent assay (ELISA) [2,20–22,24], chemiluminescent microparticle immunoassay (CMIA) [23], complement fixation test (CFT) [2], and enhanced chemiluminescence (ECL) [5]. Two studies did not mention the serological detection methods for the diagnosis of *T. gondii* [1,19]. The characteristics of the studies included in the meta-analysis of the relationship between *T. gondii* and ASD are presented in Tables 1 and 2.

On the basis of the meta-analysis results, the OR of the risk of anti-

Table 1
Characteristics of the included studies to investigate the association between *T. gondii* infection and ASD.

No	First author	Publication year	Place of study	Type of study	Diagnostic method for *Toxoplasma*	Type of antibody	Age (years±SD)	Sex (n)
1	Grether et al. [13]	2010	United States	Case control	EIA	IgG, IgM, IgA	P: <70 C: <70	P: (F:33, M:180)C: (F:44, M:221)
2	Ververi et al. [19]	2012	Greece	Cross sectional	–	–	1.5–9	M: 170 F: 52
3	Afsharpaiman et al. [20]	2013	Iran	Case control	ELISA	IgG, IgM	P: 6.00 ± 2.71C: 5.67 ± 3.09	P: (F:6, M:34)C: (F:23, M:17)
4	Prandota et al. [21]	2015	Egypt	Case control	ELISA	IgG	P: 6.1 ± 2.2C: 6.1 ± 2.2	P: (F:5, M:41)C: (F:5, M:45)
5	Shehata et al. [22]	2016	Egypt	Cross sectional	ELISA	IgG, IgM	16.84 ± 7.021	-.
6	Spann et al. [23]	2016	Finland	Nested case-control	CMIA	IgG, IgM	P: <70 C: <70	P: (F:189, M:685) C: (F:189, M:685)
7	Grether et al. [24]	2016	United States	Case control	ELISA	IgG, IgM	P: <70 C: <70	P: (F:11, M:73) C: (F:20, M:139)
8	Flegr and Escudero [2]	2016	Czech Republic	Cross sectional	CFT, ELISA	IgG, IgM	P:M: 34.0 ± 10.5 F: 36.5 ± 12.3C: M: 34.8 ± 12.7 F: 32.4 ± 11.0	P: (F:19, M:21) C: (F:871, M:333)
9	Flegr and Horáček [1]	2018	Czech Republic	Cross sectional	–	–	M: - F: -	–
10	Esnafoglu et al. [5]	2017	Turkey	Case control	ECL	IgG, IgM	P: 7.63 ± 3.46C: 7.47 ± 3.40	P: (F:17, M:85) C: (F:14, M:37)

EIA: enzyme immunoassays, ELISA: enzyme-linked immunosorbent assay, CMIA: chemiluminescent microparticle immunoassay, CFT: complement fixation test, ECL: enhanced chemiluminescence: IgG: immunoglobulin G, IgM: immunoglobulin M, P: patient, C: control, F: female, M: male, and N: number.

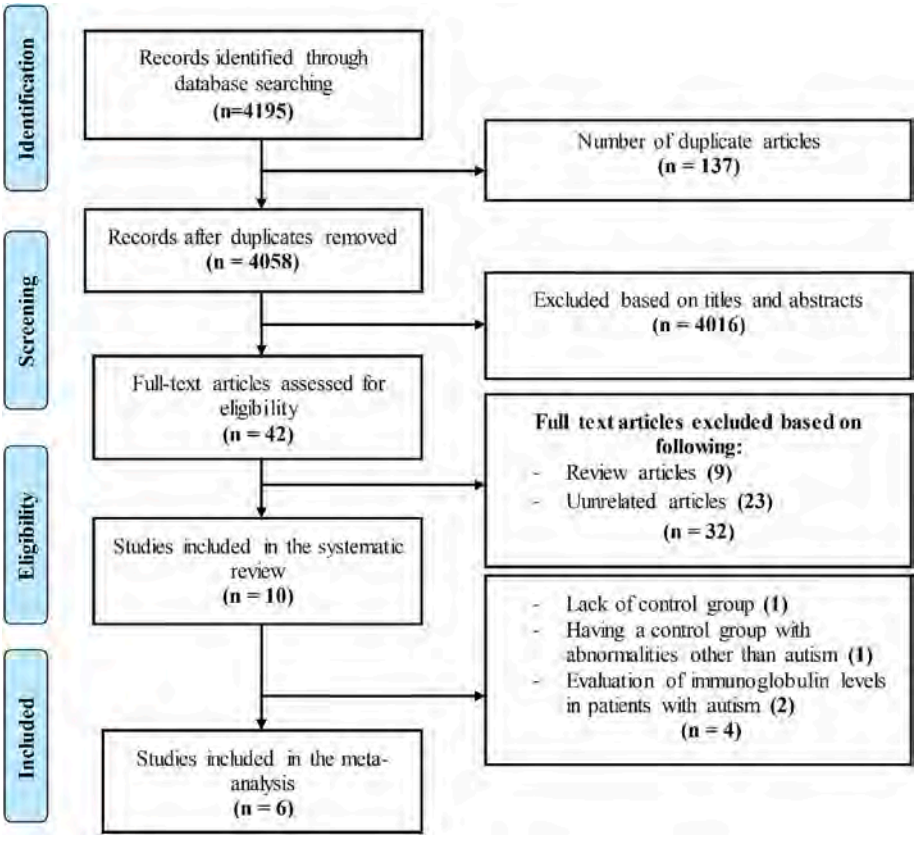

Fig. 1. Flow diagram of literature selection.

T. gondii IgG antibody in ASD patients compared to control group was 1.93 (95% CI: 1.01–3.66) (Fig. 2). The obtained results of Begg's and Egger's tests were not statistically significant (P = 0.851 and P = 0.297, respectively), indicating no publication bias. On the other hand, the graph of the funnel plot shows no publication bias (Fig. 3). The significant results of the test of heterogeneity confirmed a high level of heterogeneity (P = 0.000, I^2 = 78.1%). Statistical analysis indicated that the pooled OR of the risk of anti-*T. gondii* IgG antibody in autism patients based on the type of studies were 1.66 (95% CI: 0.54–5.09) in case-control and 2.48 (95% CI: 1.56–3.93) in cross-sectional studies (Fig. 2). Also, the OR of the risk of anti-*T. gondii* IgM antibody in ASD patients was measured at 0.39 (95% CI: 0.18–0.87) (Fig. 4). After the removal of each study, sensitivity analysis showed that the overall results were stable and strong (Fig. 5). Gender was the only patient characteristic analyzed in the current study among other data. According to the random effect models, the estimated combined ORs were 2.63

Table 2
Description of data extracted from the included studies in the systematic review and meta-analysis of the association between *T. gondii* infection and ASD.

No	First author	Total (n)	Case: ASD+ (n)	Control: ASD- (n)	ASD+ & T+ (n, %)	ASD- & T+ (n, %)	OR (95% CI)	P-value
1	Grether et al. [13]	478	213	265	–	–	–	–
2	Ververi et al. [19]	222	222	–	1 (0.45%)	–	–	–
3	Afsharpaiman et al. [20]	80	40	40	0 (0%)	1 (2.5%)	0.33 (0.01–8.22)	P = 0.31
4	Prandota et al. [21]	96	46	50	11 (23.9%)	2 (4%)	7.54 (1.57–36.20)	P < 0.0043
5	Shehata et al. [22]	6	6	182	3 (50%)	91 (50%)	…	P = 0.034
6	Spann et al. [23]	1748	874	874	210 (24.03)	198 (22.65)	1.08 (0.87–1.35)	P = 0.9
7	Grether et al. [24]	243	84	159	–	–	–	–
8	Flegr and Escudero [2]	1244	40	1204	14 (35%)	276 (23%)	1.81 (0.93–3.52)	P = 0·008
9	Flegr and Horáček [1]	23224	92	23132	33 (35.87%)	3682 (15.92%)	2.95 (1.93–4.53)	P = 0.008
10	Esnafoglu et al. [5]	153	102	51	3 (2.9%)	1 (2%)	1.52 (0.15–14.94)	–

N and n: number, CI: confidence interval, ASD^{+}: people with autism spectrum disorders, ASD^{-}: people without autism spectrum disorders, ASD^{+} & T^{+}: people with autism spectrum disorders and *Toxoplasma* positive, ASD^{-} & T^{+}: people without autism spectrum disorders and *Toxoplasma* positive, OR: odd ratio.

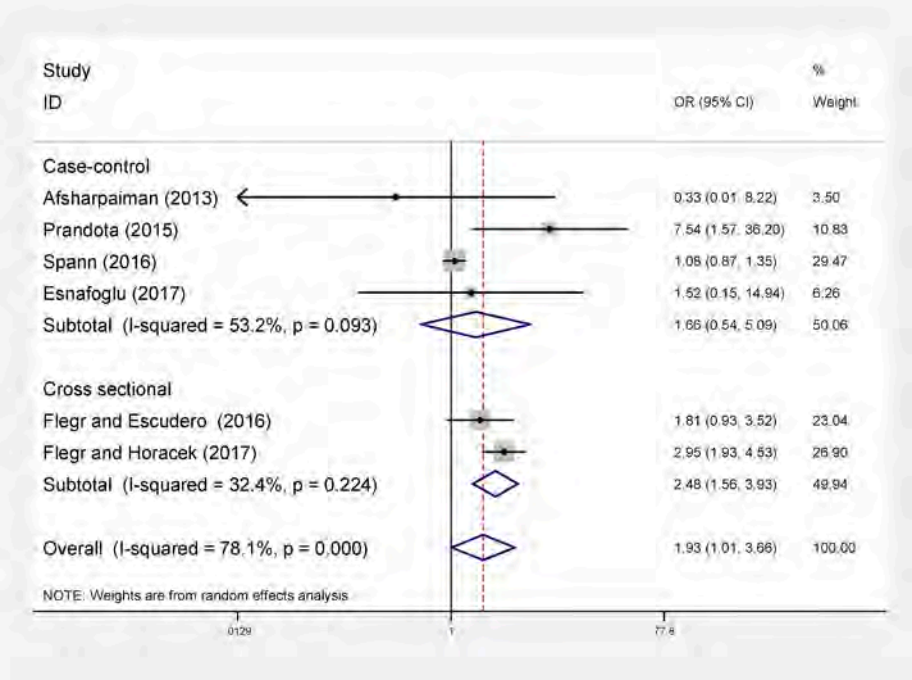

Fig. 2. Forest plot of association between IgG seropositivity rates of *Toxoplasma gondii* and risk of autism. The perpendicular continuous line represents the null hypothesis. The horizontal lines illustrate 95% CI for OR. A study that its CI (horizontal line) interrupts the vertical continuous line (line null hypothesis), the odd ratio of this study is not statistically significant.

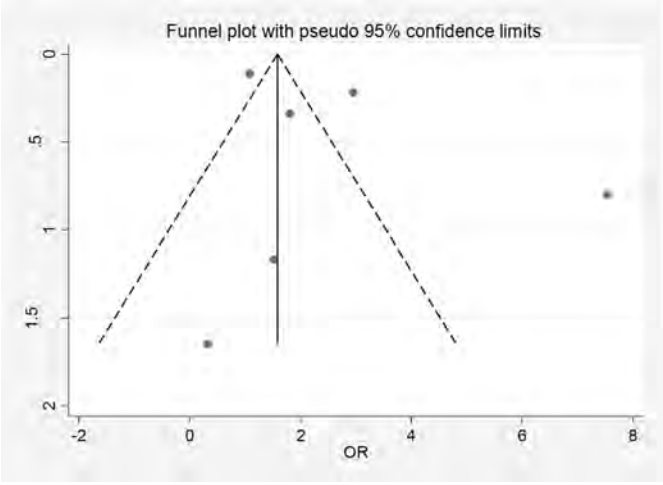

Fig. 3. Evaluation of publication bias with funnel plot.

(95% CI: 0.29–23.63) and 2.62 (95% CI: 0.94–7.30) in female and male participants, respectively. The results of the meta-regression test showed that the type of study had no significant effect on the heterogeneity of the studies (P = 0.895).

Based on the NOS checklist, in the case-control studies, the scores of 3, 4–6, and 7–9 were representative of low, moderate, and high quality, respectively. Furthermore, regarding the cross-sectional studies, the scores of 1–2, 3–5, and 6–7 indicated low, moderate, and high quality, respectively. The quality scores of different eligible studies are represented in supplementary 3.

4. Discussion

The *T. gondii,* as an important opportunistic pathogen, is one of the reasons for mental disorders [26]. However, the association of *T. gondii* infection and ASD is largely unknown. Therefore, the current systematic review and meta-analysis aimed to summarize the current evidence that may associate toxoplasmosis with ASD. Although few studies were included in our meta-analysis, the global OR from six studies for latent toxoplasmosis was estimated at 1.93 with a 95% CI of 1.01–3.66, which was indicative of a positive association between the prevalence rate of

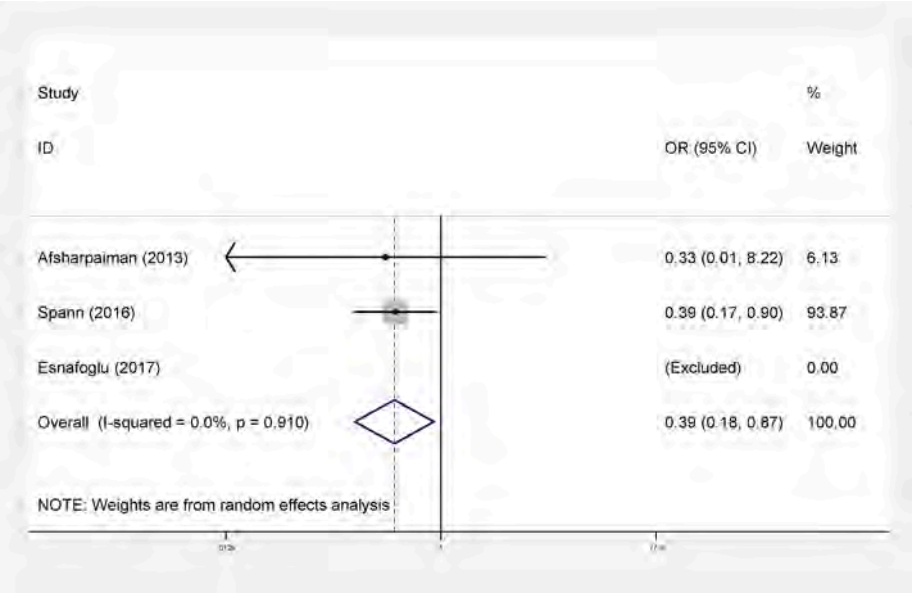

Fig. 4. Forest plot of association between IgM seropositivity rates of *Toxoplasma gondii* and risk of autism.

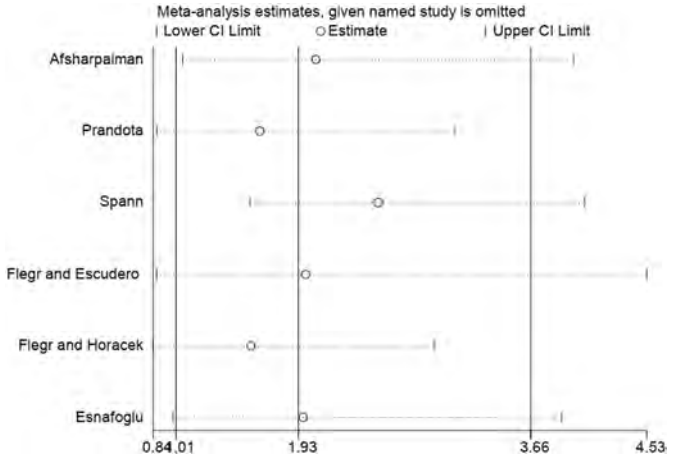

Fig. 5. Sensitivity analysis for assessing the effect of each primary study on the total estimates.

toxoplasmosis and ASD. In the current study, the overall OR of anti-*T. gondii* IgM antibody in patients with ASD compared with the control group was estimated at 0.39 (95% CI: 0.18–0.87). The number of IgM positive individuals was reported in only three studies. Considering that in one study, the outcome was zero in the case and control groups, so this study was not analyzed. Two other studies were analyzed and the results of the analysis of these two studies showed an OR < 1, which was indicative of the protective effect of *T. gondii* on ASD. The small number of studies limits the reliability of findings, and more studies are needed to provide more reliable estimates. Furthermore, we found high heterogeneity in the association between autism and *T. gondii* infection in this systematic review and meta-analysis. There are several sources for heterogeneity. In case-control studies, selection of cases and controls in different populations and in different ways have been done. Moreover, different ways for the selection of the target groups can be a source of heterogeneity. The age of the participants in the studies is another source of heterogeneity; because, advanced age increases the chance of exposure to *Toxoplasma*. The type of study may also be a heterogeneous source. Since age and gender cannot be matched in cross-sectional studies, this may be one of the reasons for the presence of heterogeneity in studies. Nevertheless, in our study, the results of the meta-regression test indicated that the type of study does not have a significant effect on the heterogeneity of the studies (P = 0.895). In addition, the included articles in the present meta-analysis showed an acceptable quality (i.e., >2 for cross-sectional studies and >3for case-control studies). A higher score from NOS checklist indicates a higher quality of a study. In fact, this study considered most of the effective items in quality. Inconsistent with our results, some studies reported that *T. gondii* was involved in obsessive-compulsive disorder (OR = 1.96) [6], epilepsy (OR = 1.62) [8], rheumatoid arthritis (OR = 3.30) [10]. However, other studies reported no significant correlation between *Toxoplasma* and multiple sclerosis (OR = 0.72) [11], Parkinson's disease (OR = 1.17) [7], and depression (OR = 1.15) [9], which contradicted with the obtained results of the current study.

The six articles included in the present meta-analysis were conducted in three continents of Asia, Europe, and Africa. Considering the settings of the articles, three studies were in Europe [Czech and Slovak (2), and Finland (1)], two in Asia [Turkey (1) and Iran (1)], and one in Africa [Egypt (1)]. However, an evident information gap was identified for the continents of America and Australia and many other countries, where there was no available data on the relationship between *T. gondii* and ASD.

The incidence of ASD has increased dramatically among children in developed countries over the last two decades [27]. Among various causes attributed to ASD, genetic factors are the dominant ones with the estimated heritability of 64–91%. Although ASD is recognized as a genetic disorder, no gene is yet identified as the agent. Therefore, it seems that genetic factors, as well as their interactions with other biological or environmental factors, may play a key role in the occurrence of ASD. The *T. gondii* interactome genes were significantly enriched in the susceptibly gene datasets for all diseases. For instance, the susceptibility genes in autism are 1117 genes among which 142 genes (12.7%) are involved in *T. gondii* interactome [5,28].

It has been shown that about one-third of spontaneous, non-inherited genetic mutations in ASD patients are found in the general population [12]. As reported, changes in the integrity of the gut barrier may be important in the genesis of ASD. Furthermore, the occurrence of ASD can be attributed to environmental, ethnic, cultural, and socioeconomic factors. Additionally, parental age, low birth weight, multiple births, and mode of birth [12], as well as intrauterine infections and immune imbalances [29] have also been identified as other risk factors involved in ASD. Changes in enteric pathogens, diet, antibiotic usage, and

reduction in the numbers and types of different bacteria caused changes in gut microbes [30,31]. In this regard, few studies have examined the association between foodborne intestinal parasites and ASD. Halliez and Buret reported a range of foodborne parasites, such as *Cryptosporidium parvum*, *Trypanosoma cruzi*, *Entamoeba histolytica*, *Giardia duodenalis*, *Hymenolepis diminuta*, *Trichinella spiralis*, and *T. gondii*, may alter gastrointestinal motility, absorption, and/or secretion via the enteric nervous system [32]. Among the foodborne parasites, *Toxoplasma* is the only parasite that has been investigated for its possible association with autism. The reason for some psychiatric disorders in humans, such as Parkinson's disease and Alzheimer's disease, is the ability of *T. gondii* to alter humoral and cellular immune responses and neurotransmitters, including dopamine [33].

The macrophages, T lymphocytes, natural killer cells, and the cytokines, are the main elements involved in immune responses. However, antibodies play a small role in controlling the infection of *T. gondii* [34,35]. Patients with autism were diagnosed with an increased level of dopamine. Testosterone hormone is associated with this neurotransmitter and both are increased during latent toxoplasmosis. As a result, the elevated level of testosterone increases the level of dopamine. In addition, nitric oxide [21] and other inflammatory cytokines, such as Interleukin-2 (IL- 2) and IL-6 increase dopamine release. Stibbs reported that the concentration of dopamine in the brains of mice with chronic toxoplasmosis is 14% higher than that of the control group [36]. Persistent neuroinflammation and hypercytokinemia are seen in patients with autism [37]. Cytokines are involved in innate immunity against *T. gondii* infection [29]. The control of acute and chronic phases of *T. gondii* infection depends on the production of proinflammatory cytokines by macrophages and lymphocytes [38].

One of the variables that should be considered in the study of the association between *Toxoplasma* and autism is gender. Based on the results of the sub-group analysis for gender, the risk of *T. gondii* infection in women and men with autism are not statistically different. The extreme male brain theory of autism is related to the preponderance of autism among males and different theories about gender differences [39]. Moreover, it was proposed that an increased prenatal level of testosterone could be related to both latent toxoplasmosis and autism; moreover, an increased concentration of testosterone was observed in men with latent toxoplasmosis, compared to negative toxoplasmic subjects. Autism affects females less frequently than males [29]. The limitations of the investigated studies in the present meta-analysis include the selection of case and control groups as one of the major problems in case-control studies. In one study, Ververi *et al.* did not mention the diagnostic method of *T. gondii*. However, because it examined congenital toxoplasmosis in children with autism was eligible to include in this systematic review. This study was not analyzed due to the lack of a control group [19]. Another study involved a large population and individuals were invited to participate in the study through the snowball sampling technique using Facebook and electronic media. The information provided was not based on medical records and the people reported their health status. In addition, there may be incorrect data. Nevertheless, since this study presents all the positive and negative results, this approach solves drawer problems and the tendency to publish positive results of studies [1]. On the other hand, Flegr *et al.*, in another study showed that information about toxoplasmosis was correct in 99.5% of individuals who were tested in the laboratory for *Toxoplasma* seropositivity and later registered as internet volunteers [40]. Due to the large sample size and other cases mentioned, the results of this study are valid and reliable. Therefore, this study was analyzed. In one of these studies, the participants were enrolled to evaluate the relationship between blood group with personality, performance, and health. Therefore, there is a possibility of the presence of participants with health problems and this part of the results cannot be generalized to the whole population [2]. Furthermore, the lack of data related to longitudinal follow-up of infected patients and the time of infection when the serum sample was drawn can be considered as another shortcoming.

This systematic review has several limitations as follows: (1) few numbers of studies that investigated the relationship between *T. gondii* infection and ASD, (2) different diagnostic methods with various specificities and sensitivities were used to measure the levels of IgG and IgM anti-*T. gondii* antibodies, (3) in most case-control studies, various variables (including age, sex, education level, etc.) did not have the exact matching, and (4) lack of the evaluation of risk factors, such as parental age, low birth weight, multiple births, and mode of birth.

5. Conclusion

Based on the results of this meta-analysis, it can be concluded that a statistically significant association was observed between *T. gondii* infection in cases with autism and control groups. Therefore, future investigations are needed to answer any questions. Future studies are required to elucidate the developmental pathways to this disorder, the relationship between maternal immune activation and *T. gondii* in the observed associations with childhood autism, evaluation of immunoglobulins and other immune markers, and possible effect modification associated with gene-pool factors or geographic differences in maternal exposure.

Ethical approval

The code of ethics of this plan is (IR. MAZUMS.REC.1398.795).

Informed consent

All authors approved the final version of the manuscript.

Declaration of competing interest

None declared.

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This article is an approved plan from Student Research Committee of Mazandaran University of Medical Sciences, Sari, Iran (number: 5504).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2020.104434.

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