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SHORT COMMUNICATION

Toxoplasma gondii Infection Among Institutionalized Children with Down syndrome in Sana'a city, Yemen: Implications of Low IgG Seroprevalence

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Abstract

Purpose To assess the IgG seroprevalence of *Toxoplasma gondii* as an indicator of past exposure and immunity against infection among children with Down syndrome (DS) in Sana'a city, Yemen. This preliminary study is justified by the primary immunodeficiency of children with DS and the opportunistic nature of the parasite, considering the vague situation of anti-*Toxoplasma* IgG seroprevalence among children with DS because of neglecting its study on local and global scales.

Methods This descriptive, facility-based, cross-sectional study was conducted among 107 children with DS hosted in six randomly selected rehabilitation centers for children with special needs in Sana'a city. Demographics of children and their mothers' knowledge of toxoplasmosis were collected using a pre-designed, structured questionnaire. Anti-*Toxoplasma* IgG antibodies were measured in the sera of children using electrochemiluminescence assay.

Results Of 107 children with DS, 3 (2.8%) were seropositive for anti-*Toxoplasma* IgG. Approximately two-thirds (71/106) of the mothers of children with DS were aware of toxoplasmosis. Of whom, 83.1% (59/71) were aware of its congenital complications.

Conclusion The majority of children with DS in Sana'a city are seronegative for anti-*Toxoplasma* IgG, where the seropositivity rate is lower than 3.0%. Therefore, children with DS are non-immune and susceptible to the acquisition of primary infections during their life. Further analytical studies are recommended to determine whether the defective immune response of children with DS is associated with false seronegativity, to assess the role of their mothers' knowledge in reducing their exposure to infection if they were confirmed truly seronegative and to identify the predictors of infection among them.

Keywords *Toxoplasma gondii* · Seroprevalence · Down syndrome · Immunodeficiency · Yemen

Introduction

Toxoplasma gondii is an obligate, opportunistic intracellular apicomplexan parasite with a complex life cycle [1]. It chronically infects about 30–50% of the human population worldwide [2]. Acquired primary infection with *T. gondii* is usually asymptomatic in immunocompetent people, but patients may experience fever, cervical lymphadenopathy and retinochoroiditis with nonspecific clinical signs [2]. However, chronic infection with *T. gondii* may affect human behaviors, cognitive functions, cryptogenic epilepsy, headaches and schizophrenia [3, 4]. *T. gondii* can cause severe and life-threatening cerebral complications in immunocompromized patients, including those with acquired immunodeficiency syndrome, cancer patients undergoing chemotherapy and organ transplant recipients. Primary infection during pregnancy can lead to congenital toxoplasmosis with severe complications in the fetus or newborn infant, including brain or eye damage, spontaneous abortion, stillbirth, or even death [5].

Down syndrome (DS), or trisomy 21, is one of the most common chromosomal anomalies, with an incidence of 1 per 1000 live births worldwide [6]. It is characterized by

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mental retardation and impaired cognitive functions, characteristic facial features, and defects in the brain, heart and gastrointestinal tract [7, 8]. In addition, it is the most common genetic syndrome associated with immune defects [9, 10]. Therefore, children with DS are at high risk of infection and severe complications caused by opportunistic pathogens, including *T. gondii* [11]. However, there is a lack of studies on the seroprevalence of *T. gondii* infection among children with DS on a global scale. In Yemen, one of the least-developed countries, a few studies have been conducted on *T. gondii* infection among different population categories in different areas, mainly focusing on the epidemiology of infection among pregnant women. Therefore, the present pilot study assessed the immune status of children with DS against infection with *T. gondii* by determining anti-*Toxoplasma* immunoglobulin G (IgG) seroprevalence among institutionalized children with DS in Sana'a city, Yemen.

Materials and Methods

This descriptive, facility-based, cross-sectional study was conducted among children with DS registered in six randomly selected rehabilitation centers for children with special needs in Sana'a from November 2019 to April 2020. The children were hosted in the institutions on a daily basis, except for the weekends and official vacations, to receive physical and mental rehabilitation besides special educational interventions. They were identified by their identity cards besides their physical and facial features and were recruited if they gave assent and cooperated adequately during sample collection besides obtaining written informed consent of their parents/legal representatives. It was ensured that none of the recruited children had detrimental systemic diseases, such as malignancies and type 1 diabetes mellitus, or any other immunocompromizing conditions. A minimum sample size of 104 children was calculated using OpenEpi, Version 3.01 (http://www.openepi.com) based on the following parameters: DS population of 250 registered children, a 95% confidence level, an expected seroprevalence of 13.3% according to the single available estimate of *T. gondii* seroprevalence among Egyptian children with DS [12] and an accepted marginal error of 5.0%. However, 107 children with DS were included in the study. Although simple random sampling was planned to select children from the sampling frame of those registered in the centers, this sampling strategy was not possible due to the absenteeism of most children during the coronavirus disease 2019 (COVID-19) pandemic. Therefore, consecutive sampling of children with DS who met the inclusion criteria was adopted.

Demographic characteristics, including gender, age, residence, household size, maternal education and employment status, were collected using a pre-designed, structured questionnaire. In addition, data about mothers' knowledge of toxoplasmosis and its congenital complications were also collected. In addition, 3–5 ml of blood were collected by aseptic venipuncture into pre-labeled plain test tubes and left to clot at room temperature. Sera were then separated, transferred into pre-labeled Eppendorf tubes and stored at − 20 °C until serological testing. Anti-*Toxoplasma* IgG antibodies were measured in international units (IU)/ml using Elecsys® Toxo IgG kits (Roche Diagnostics GmbH, Mannheim, Germany) with automated electrochemiluminescence assay (ECLIA) by cobas® e 411 immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany) according to the measurement protocols of the manufacturer. Sera with titers < 1 IU/ml were considered non-reactive, sera with titers ≤ 1 IU/ml to < 3 IU/ml were considered indeterminate and sera with titers ≥ 3 IU/ml were considered reactive. According to the manufacturer, the relative sensitivity of this immunoassay ranges from 99.5 to 100.0% [lower 95% confidence interval (CI): 97.6–99.1%], and the relative specificity of the immunoassay ranges from 98.8 to 100.0% [lower 95% confidence interval 95% CI: 96.8–99.2%] [13]. Moreover, a recent systematic review and meta-analysis of assays for detecting anti-*Toxoplasma* IgG revealed a mean sensitivity level of 98.6% (95% CI: 97.5–100), a mean specificity level of 99.6% (95% CI: 98.7–100), a mean positive predictive value of 99.2% (95% CI: 98.3–100) and a mean negative predictive value of 99.4% (95% CI: 98.7–100) for Elecsys® Toxo IgG assay, with 100% concordance with the dye test for sera with interfering diseases [14].

Data were then analyzed using the IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies and proportions, while continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data or median ± interquartile range (IQR) for non-normally distributed data.

Results

Table 1 shows that more than half of children with DS were males (56.1%) and aged ≤ 10 years (54.2%), with a median age of 10.0 ± 6.0 years (range: 2.0–15.6). The majority of children (91.6%) were urban residents and living within households of seven members or more (57.6%). On the other hand, the majority of the mothers of children with DS were older than 35 years (83.3%), literate (54.7%) and unemployed (86.8%). Approximately two-thirds (71/106) of the mothers of children with DS were aware of toxoplasmosis. Of whom, 83.1% (59/71) were aware of its congenital complications. Figure 1 shows that 3 of 107 (2.8%) children with DS were seropositive for anti-*Toxoplasma* IgG.

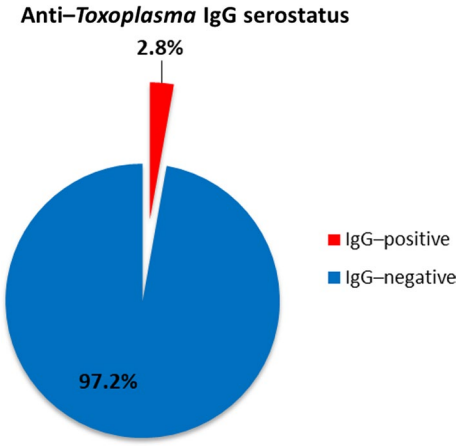

Fig. 1 Seroprevalence of anti-*Toxoplasma* IgG among children with DS in Sana'a city, Yemen (2019–2020)

Discussion

To the best of our knowledge, this is the first study to report on *T. gondii* infection among children with DS in Yemen. Children with DS have abnormal innate and adaptive immune systems [11]. Yet, most studies about infectious diseases among this immunocompromized population category focused on respiratory tract infections [15–18] but neglected *T. gondii* infection. The lack of studies on the seroprevalence of anti-*Toxoplasma* IgG as an indicator of the past exposure and immune status of children with DS against the infection on the global and regional scales prompted us to conduct this descriptive study to form a basis upon which further analytical studies can be designed. The seroprevalence of anti-*Toxoplasma* IgG among children with DS in Sana'a city was less than 3.0%, showing that children with DS are non-immune to the parasite and are at potential risk of primary infection during their life, which can lead to serious and life-threatening consequences. Several scenarios are raised to interpret such a low anti-*Toxoplasma* IgG seropositivity rate, which entail the need for testing them by further analytical studies.

One scenario behind such low seroprevalence could be the weak humoral immune response in children with DS that cannot mount to produce detectable levels of antibodies. In this respect, specific antibody responses have been found to be lower in children with DS than normal ones after exposure to various vaccines [19]. The abnormalities in IgG titers do not occur in all DS patients, where some can have under-normal titers while others show adequate titers [19, 20]. The impaired humoral immune response of children with DS could lead to false-negative IgG results, which might appear as non-reactive or indeterminate using the technique adopted in this study. In such a case, children with DS can be exposed to recurrent *T. gondii* infections because of the lack of sufficient immune response after exposure to infection. Consequently, the high IgG seronegativity rate of *T. gondii* infection among children with DS in the present study does not exclude their past exposure to infection.

IgG seroprevalence rate among children with DS in the present study is lower than that (13.3%) recently reported among Egyptian children with DS in Mansoura governorate [12]. It is also lower than that reported among apparently healthy children (16.0%; 40/250) and children with visual and/or hearing disabilities (32.5%; 39/120) in Taiz

Table 1 Characteristics of children with DS and their mothers in Sana'a city, Yemen (2019–2020)

Characteristics	*n*	(%)
Gender		
Male	60	(56.1)
Female	47	(43.9)
Age (years)		
< 10	49	(45.8)
≥ 10	58	(54.2)
Median ± IQR (range) 10.0 ± 6.0 (2.0–15.6)		
Residence		
Rural	9	(8.4)
Urban	98	(91.6)
Household size (members)[a]		
< 7	39	(42.4)
≥ 7	53	(57.6)
Median ± IQR (range) 7 ± 4 (3–12)		
Mother's age (years)[b]		
≤ 35	17	(16.7)
> 35	85	(83.3)
Mean ± SD (range) 43.2 ± 7.7 (23.0–60.0)		
Mother's literacy status		
Illiterate	48	(45.3)
Literate [c]	58	(54.7)
Mother's employment status		
Unemployed	92	(86.8)
Employed	14	(13.2)
Mother's knowledge of toxoplasmosis		
Yes	71	(67.0)
No	35	(33.0)
Mother's knowledge of congenital toxoplasmosis complications[d]		
Yes	59	(83.1)
No	12	(16.9)

DS Down syndrome

The number of children with DS enrolled in the study was 107, while the number of the mothers of children with DS who responded to the questionnaire was 106 (one was dead)

[a]Missing for 15 children

[b]Missing for four mothers

[c]Receiving informal or formal education and can read and write

[d]Calculated for mothers aware of toxoplasmosis

city, southwest of Yemen [21]. Although differences in sociodemographic characteristics, cultural habits, eating habits, environmental factors and using different antibody detection techniques are among the several factors leading to variations in anti-*Toxoplasma* IgG seroprevalence, the impaired immune responses of children with DS could play a role in this concern and make it difficult to compare them to children with apparently normal immunity or with other types of immunocompromizing conditions. For instance, higher IgG seroprevalence rates of 41.7% and 36.6% were reported for infection with *T. gondii* among Iranian children with cancer and hematological malignancies, respectively [22, 23]. Anti-*Toxoplasma* IgG seroprevalence among children with DS in the present study is also lower than that (18.7–43.7%) reported among pregnant women seeking healthcare in Sana'a city [24, 25]. It is noteworthy that age of 25 years or older, illiteracy, living within large households, rearing cats indoors and contact with soil had been reported as significant risk factors for infection with *T. gondii* in the city [24, 25].

Another scenario behind the low anti-*Toxoplasma* IgG seroprevalence among children with DS could be partially attributed to the fact that the majority of mothers were aware of toxoplasmosis. The more attention given to such children, their almost indoor stay and inaccessibility to playing outdoors compared to ordinary children can reduce their environmental and dietary exposure to infection. Given that two-thirds of the mothers of children with DS in the present study were aware of toxoplasmosis and more than 80.0% of knowledgeable mothers perceived the fetal complications of congenital toxoplasmosis, the role of such knowledge in reducing the exposure of children to infection sources could not be ruled out. However, the association of the absence of such knowledge with anti-*Toxoplasma* IgG seropositivity could not be tested in the present study because of the low seropositivity rate. In contrast to the present study, 46.9% and 44.7% of pregnant women in Sana'a city were reported to be aware of toxoplasmosis and its modes of transmission, respectively [24]. In another context, a lower proportion (11.0%) of pregnant women from Malaysia, the Philippines and Thailand were reported to have heard of toxoplasmosis [26]. One explanation for such knowledge difference is that the mothers of children with DS realize the defective immunity of their children and tend to educate themselves about the infections affecting them. When asking the mothers about their knowledge of any pathogen of importance during pregnancy, they confirmed their knowledge of a "cat germ" that can cause miscarriage, blindness, or congenital anomalies to the fetus. The "cat germ" is their locally rolling expression to refer to "*T. gondii*".

This study is limited by being purely descriptive. Notwithstanding, its findings as a preliminary study on a neglected infection among children with DS provide insights into the susceptibility of such children to infection with *T. gondii* and its severe consequences and generate hypotheses about the reasons behind the high proportion of children non-immune to infection. Besides, it opens new horizons for further analytical studies about this opportunistic infection among children with DS. Another limitation could be introduced by conducting the study in facilities hosting children with DS, discrediting the generalizability of its findings to children with DS at the community level, particularly in rural areas where such facilities are not accessible. Apart from the immunological aspect of the infection among children with DS, it is imperative to explore the behavioral and environmental factors that could play a role in their exposure to infection within and outside the hosting institutions.

The primary immunodeficiency caused by DS and seronegativity for *T. gondii* infection can pose the children with DS to severe complications if primarily infected with the parasite during their life. Therefore, regular screening of children with DS for anti-*Toxoplasma* IgM and, whenever possible, detecting the infection as early as possible using molecular or antigen-based techniques are recommended. Meanwhile, it is imperative to develop and implement mother- and facility-oriented health education interventions to raise the awareness of mothers and caregivers in the institutions and centers hosting such children about the sources of infection, risk factors and the best preventive practices against infection with *T. gondii* among children with DS.

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Availability of data Data of this study are provided in the paper.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The protocol of this study was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences, Sana'a University, Sana'a, Yemen. Moreover, permission was obtained from the directors of the rehabilitation centers for children with special needs selected for the study.

Consent to participate Written informed consent was obtained from parents/legal representatives of children with DS before data and sample collection after explaining to them the aim and procedures of the study.

Consent for publication Not applicable.

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