Toxoplasma and Toxocara seropositivity in juvenile idiopathic arthritis and its relation to disease activity and type of therapies

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ABSTRACT

Juvenile idiopathic arthritis (JIA) is the most frequently encountered autoimmune rheumatic disease in children. To our knowledge, this is the first study aimed to estimate the frequency of Toxoplasma gondii (T. gondii) and Toxocara seropositivity in JIA and assess its relation to the disease activity, IL-10 levels, and type of the received therapies. This study was conducted on 43 JIA patients and 50 cases as a control group. All participants were evaluated by disease activity score (JADAS-27), and the presence of specific IgG and IgM antibodies against T. gondii and IgG against Toxocara species using an enzyme-linked immunosorbent assay. IL-10 serum levels were measured using an ELISA kit. The results show that JIA patients have significantly higher seropositivity for anti-T. gondii IgG compared to control subjects ($p = 0.02$) and a non-significant difference for Toxocara seropositivity ($p = 0.41$). All participants were negative for IgM anti-Toxoplasma gondii. Demographic parameters did not significantly affect these seroprevalence frequencies ($p > 0.05$). IL-10 was significantly higher among JIA patients compared to controls ($p = 0.007$) and seropositive anti-T. gondii JIA exhibited significantly higher IL-10 levels compared to seronegative ones ($p = 0.03$). Seropositive anti-T. gondii IgG JIA patients had a significantly higher disease activity score (JADAS-27) than seronegative anti-T. gondii IgG cases ($p = 0.02$). There was a significant positive correlation between anti-T. gondii IgG and JADAS-27 score ($p = 0.009$). A significant association was detected between T. gondii infection and DMARDs including the biological therapies ($p < 0.05$). Overall, this study supports a possible association between T. gondii infection and JIA, IL-10, disease activity score, and DMARDs therapies. It is possible that IL-10 plays a role in the development of JIA and contributes to persistent asymptomatic infection with T. gondii in JIA patients. As a result, a recommendation for screening tests for T. gondii infection among JIA patients is crucial before and during commencing DMARDs therapies and closely monitoring early signs of infection.

1. Introduction

The prevalence of autoimmune diseases is increasing worldwide at an alarming rate and is a threatening public health problem such

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as cancer and heart disease. Juvenile idiopathic arthritis (JIA) is the fifth most common chronic illness of childhood and the most prevalent chronic rheumatologic disease that cause inflammation of the joints and disability with a significant socioeconomic impact. It is characterized by arthritis for at least 6 weeks of unknown etiology that begins before the age of 16 years (Hyrich et al., 2010; Hahn and Kim, 2021).

The etiology of JIA is still poorly understood; JIA is a multifactorial condition related to genetic background and environmental factors. Some studies reported a link between *Toxoplasma gondii* (*T. gondii*) and *Toxocara* spp. infections and a variety of autoimmune diseases, including rheumatoid arthritis (RA). The severity of arthritis is associated with *Toxocara canis* infections as the parasite induces the secretion of pro-inflammatory cytokines like IL-33 (Gallardo et al., 2016) and Hosseininejad et al. (2018) in a meta-analysis reported that *T. gondii* infection possibly causes and exacerbates the symptoms of RA. In addition, anti-*Toxoplasma*-antibodies are more prevalent in other autoimmune disorders such as systemic sclerosis, primary and secondary antiphospholipid antibody syndrome, primary biliary cirrhosis, autoimmune thyroid diseases, pemphigus vulgaris, and autoimmune vasculitis (Petríková et al., 2010).

The World Health Organization considers toxoplasmosis and toxocariasis as neglected tropical diseases despite the relevance of the illnesses among the high-risk population including patients with autoimmune diseases. As an apicomplexan protozoan, *T. gondii* is an obligate intracellular pathogen. It is estimated that approximately one-third of the world's population is infected with this pathogen in developed and developing countries (Dubey et al., 1998; Jones et al., 2008). Humans acquire the infection by ingestion of raw or undercooked meat containing tissue cysts or contact with cat feces from the soil and by ingesting food or water contaminated with sporulated oocysts (Hill and Dubey, 2002). Toxoplasmosis may cause polytenosynovitis (inflammation of a tendon sheath) and polyarthritis in the hand and knee joints (Vass et al., 1977; Balleari et al., 1991).

Toxocariasis is a neglected parasitic zoonosis that affects millions of pediatric and adolescent populations worldwide caused by *Toxocara canis* and *Toxocara catis*. It is of global importance, but due to a lack of clinical awareness, standardized diagnostic criteria, and coordinated epidemiological surveillance, there is a relative scarcity of understanding of this significant infection (Macpherson, 2013). Toxocariasis is a silent progressive public health threat that is acquired by ingestion of the embryonated eggs of the parasite. In humans, larvae travel through a variety of internal organs, causing neurotoxocariasis, ocular toxocariasis, or visceral larva migrans (Rubinsky-Elefant et al., 2010). Depending on the parasite burden, age, duration of larval migration, and immune-mediated response of the individual, the severity of the disease varies (Magnaval et al., 2001).

Cytokine signals play an important role in both protective immunity and immunopathology. The host response to infection necessitates a strong immune response capable of controlling the pathogen and minimizing immune-mediated disease. The immune suppressant cytokine interleukin-10 (IL-10) is produced by both leukocytes and non-hematopoietic cells. IL-10 can successfully inhibit the production of the pro-inflammatory cytokines IL-1, IL-8, and TNF-α by macrophages and synoviocytes. IL10 was also associated with increased autoantibody synthesis and B cell activation. In addition, IL-10 dramatically modifies macrophage effector functions to fight various infections (Hart et al., 1995).

A clear relationship between *T. gondii* and *Toxocara* species infection and JIA has not yet been well documented. To our knowledge, this is the first study investigating the frequency of *T. gondii* and *Toxocara* species among JIA patients and evaluating any possible association with the disease activity, IL-10, and the type of treatment received among these patients.

2. Subjects and methods

2.1. Study population and design

This is a case-control study that included 93 participants. The study was carried out between April 2020 and March 2021 and included 43 JIA patients and 50 children as a control group of age, gender-matched, and from the same geographic region. The JIA patients were enrolled at the Pediatric Rheumatology Clinic during their routine follow-up visits at Mansoura University Children's Hospital. The control group was selected randomly from children below 16 years who were referred to the Mansoura University Children's Hospital clinics for regular checking.

The inclusion criteria for JIA cases were a definite diagnosis of JIA according to the International League of Associations for Rheumatology (ILAR) (Petty et al., 2004), age below 16 years, both genders, with any duration of JIA, and agreeing to participate in the study voluntarily.

Exclusion criteria for participants were age above 16 years, diabetes mellitus, systemic lupus erythematosus, malignancy, history or current evidence of infection or comorbidities, no history of anti-parasitic drugs in the previous 3 months, hematologic disease, such as leukemia which may impact the generation of antibody responses against infection.

2.2. Ethical considerations

Ethical approval for the study was granted by the Ethical Committee of the Mansoura Faculty of Medicine-Institutional Research Board (approval number R.22.02.1619). All patients' parental consent was obtained before participation.

2.3. History and clinical assessment

Detailed medical history and complete physical examination were conducted on all participants. Medical history takes into account demographics such as age, gender, residence area, also information about contact with cats and/or dogs, and feeding habits such as raw vegetable and raw meat consumption. Clinical data (including the age of onset of JIA, duration of the disease, systemic symptoms,

number of affected joints, and type of therapy) were also obtained. The disease activity was evaluated using the JADAS-27 score. The JADAS-27 (range 0–57) final score was calculated by the sum of the scores of four components: physician's global assessment of disease activity (PGA) measured in a 10-cm visual analog scale (VAS); parent/patient global assessment of well-being also measured on a 10-cm VAS; active arthritis, defined as joint swelling or limitation of movement accompanied by pain and tenderness, assessed in 27 joints; and erythrocyte sedimentation rate (ESR) in mm/h converted to a scale from zero-10, using the formula ESR − 20/10, whereby, before the calculation, ESR values <20 mm/h were converted to 0 and ESR values >120 mm/h were converted to 120. The score ranges from 0 to 57 where 0, corresponds to total remission and 57 to maximum disease activity, and the cut-off score of 2.7 is considered for low and 6 for high disease activity (Ćalasan et al., 2014).

2.4. Laboratory tests

Approximately 5 mL of venous blood was drawn from each participant under aseptic conditions. Blood samples were stored overnight at room temperature, to allow blood clot formation, and then centrifuged at 1000 ×g for 10 min; sera were collected and stored at −20 °C. The collected serum samples were analyzed for the presence of IgG and IgM antibodies against *T. gondii* by Enzyme-Linked Immunosorbent Assay (ELISA) according to the manufacturer's instructions. The microplates were read at 450 nm using an ELISA microplate reader (BioTek; Winooski, Vermont, USA). Anti-*Toxoplasma gondii* IgG antibodies were assayed using qualitative and quantitative methods. The IgG test kit (Biokit Diagnostics Company, Spain) has reported sensitivity and specificity of 98% and 99%, respectively. *T. gondii* IgG ≥10 IU/mL is considered seropositive. A commercial ELISA kit (Trinity Biotech Company, USA) for IgM anti-*T. gondii* antibodies were used. Anti-*T gondii* IgM levels equal to or higher than 1.1 IU/mL were considered positive, and the IgM test kit has reported sensitivity and specificity of 100% and 99%, respectively. Detection of anti-*Toxocara* IgG serum antibodies was performed using an enzyme-linked immunosorbent assay (ELISA) kit (NovaTec Immunodiagnostics, Dietzenbach, Germany), and a cut-off of ≥11 IU/mL was used for seropositivity.

An enzyme-linked immunosorbent assay was used to determine the levels of IL-10 in the blood (ELISA; CLB, Pelikine Compact human IL-10 ELISA kit, Amsterdam, The Netherlands). The assay was carried out as directed by the manufacturer, and its sensitivity was 1 pg/mL. Both the intra- and inter-assay coefficients are <10%, according to manufacturer reports.

All patients had complete blood count (CBC), ESR, rheumatoid factor (RF), antinuclear antibodies (ANA), and anti-cyclic citrullinated peptide (anti-CCP) were also done in the hospital laboratory and radiological studies of the affected articulations were done. JIA patients were divided into two groups according to seropositivity of anti-*Toxoplasma* IgG and anti-*Toxocara* species IgG antibody into the seropositive and seronegative groups.

2.5. Statistical analysis

Data analysis was done using Statistical Package for Social Sciences (SPSS software version 20). The categorical data were presented as numbers and percentages. Means and standard deviations were calculated for quantitative data. Chi-square or Fisher s exact test was used to compare the association for categorical variables as appropriate. The normal distribution was assessed by using Kolmogorov-Smirnov. Continuous variables with normal distribution were presented as mean as mean ± standard deviation (SD) and skewed distributed variables as median (min-max). The comparisons between the two groups were performed with the Student-t-test for parametric data and the Mann-Whitney test for non-parametric data as appropriate. Pearson correlation coefficient was used to measure the strength of the linear relationship between two variables. Additionally, $P < 0.05$ was the statistically significant level.

3. Results

A total of 93 participants (43 patients with JIA and 50 cases as a control group) were included in this study. 30.2% of JIA were males and 69.8% were females. The mean age of JIA patients and controls was 10.35 ± 2.84 and 11.07 ± 2.62 years respectively. The

Table 1
Demographic and lifestyle data of studied juvenile idiopathic arthritis and control participants.

Parameter	JIA cases N = 43	Controls N = 50	p
Age, Mean ± SD (years)	10.35 ± 2.84	11.07 ± 2.62	0.17
Gender (number, percent)			
Males	13 (30.2%)	14 (28%)	0.82
Females	30 (69.8%)	36 (72%)	0.82
Residence (number, percent)			
Rural	28 (65.1%)	34 (68%)	0.77
Urban	15 (34.9%)	16 (32%)	0.77
Dog contact (yes)	3 (7%)	2 (4%)	0.53
Cat contact (yes)	8 (18.6%)	3 (6%)	0.06
Eating undercooked meat (yes)	2 (4.7%)	3 (6%)	0.78
Eating raw vegetables (yes)	3 (7%)	3 (6%)	0.85

Data presented as mean ± SD, or as the number [%]; N, number.
JIA, Juvenile idiopathic arthritis; SD, standard deviation.

controls were matched with the patients regarding age, gender, and residency (*P* > 0.05). The age groups of *T. gondii* seropositive JIA patients were six (35.3%) patients from 5 to 9 years, seven (41.2%) patients from 10 to 13 years, and four (23.5%) patients from 14 to 15 years. While the age groups of seronegative JIA patients were ten (38.5%) patients from 5 to 9 years, thirteen (50%) patients from 10 to 13 years, and three (11.5%) patients from 14 to 15 years. There was no significant difference between JIA patients and controls in terms of demographic and lifestyle variables, including contact with dogs and cats, and eating undercooked meat or raw vegetables (*p* > 0.05) (Table 1).

3.1. Seropositivity of Toxoplasma gondii, Toxocara species, and IL-10 levels among JIA patients and controls

Overall, 17 patients with JIA (39.5%) and 8 controls (16%) had seropositive anti-*T. gondii* IgG. The seropositivity of anti-*T. gondii* IgG antibodies were significantly more prevalent in patients with JIA in comparison with controls (*p* = 0.01). Moreover, JIA patients had a significantly higher mean value of anti-*T. gondii* IgG titer compared to controls (11.1 ± 5.9, 7.1 ± 3.4 respectively, *p* < 0.001). However, all participants were negative for IgM anti-*Toxoplasma gondii*. When the Odds ratio was applied, it revealed that the seropositivity of anti-*Toxoplasma* IgG was 3.43 folds higher among JIA patients compared to controls and this finding was statistically significant (p = 0.01(Table 2)).

The frequency of anti-*Toxocara* IgG antibodies among patients with JIA was 9.3% (4/43) and 4% (2/50) among controls with no significant differences (*p* = 0.31) (Table 2). JIA patients had a none significantly higher mean value of anti-*Toxocara* IgG titer compared to controls (6.8 ± 4.7, 5.4 ± 2.7 respectively, *p* = 0.09). The age of four cases positive for *Toxocara* ranged from 5 to 10 years. The seropositivity of anti-*Toxocara* IgG was found to be 2.46 times greater among JIA patients compared to controls when the odds ratio was applied, and this finding was statistically insignificant (p = 0.31).

IL-10 levels were significantly higher among JIA patients compared to controls (7.72 ± 1.88 vs 6.72 ± 1.61, *p* = 0.007) (Table 2).

3.2. The relationship of seropositivity of T. gondii and Toxocara with demographic data, disease activity score, and laboratory parameters among JIA patients

When the patients with JIA were divided into seropositive and seronegative for *Toxoplasma* and *Toxocara*, there was no statistically significant difference between seropositive and seronegative *Toxoplasma* and *Toxocara* JIA cases, in terms of any of the tested demographic and lifestyle variables (Tables 3 and 4).

IL-10 was significantly higher among *T. gondii* seropositive JIA patients compared to seronegative JIA patients (*p* = 0.03), however, there was no significant difference between seropositive *Toxocara* and seronegative JIA patients (*p* = 0.47). The disease activity score (JADAS-27) was significantly higher among JIA cases with *T. gondii* seropositive compared to seronegative cases (8.39 ± 1.64 vs. 4.65 ± 2.73; *p* = 0.02) (Table 5). There was a significant positive correlation between anti-*Toxoplasma gondii* IgG level and JADAS-27 score (r^2 = 0.39, *p* = 0.009) (Fig. 1).

There were significantly higher differences between JIA *T. gondii* seropositive in terms of duration of the disease, the number of joints, and ESR compared to seronegative cases (*p* = 0.001, 0.02, and 0.01 respectively) (Table 5).

However, there was no evidence of significant differences between seropositive JIA cases compared to seronegative for *T. gondii and Toxocara* in terms of other studied clinical and laboratory variables (i.e., subtypes of JIA, ANA, RF, CBC, and type of therapy) (*p* > 0.05) (Table 6).

The JADAS-27 score was non significantly higher among *Toxocara* seropositive cases compared to seronegative ones (*p* = 0.68) (Table 6) and there was no significant correlation between anti-*Toxocara* IgG level and JADAS-27 score (r^2=, 0.14, *p* = 0.37). There was a non-significant correlation between IL-10 and JADAS-27 score (r^2 = 0.11, *p* = 0.50).)

3.3. The relationship of seropositivity of T. gondii and Toxocara with the type of received therapies among JIA patients

Twenty-one JIA patients (48.9%) used biologic DMARDs therapies (eight cases were on etanercept, nine cases were on infliximab,

Table 2
Seropositivity of *Toxoplasma gondii*, *Toxocara* species and IL-10 levels among studied pariciants.

Parameter	JIA Cases N = 43	Controls N = 50	Odds ratio (95% CI)	p
Toxoplasma gondii				
Seropositive IgG, N (%)	17 (39.5%)	8 (16%)	3.43(1.29–9.16)	**0.01**
IgG titer; Mean ± SD	11.1 ± 5.9	7.1 ± 3.4		**<0.001**
Toxocara species				
Seropositive, N (%)	4 (9.3%)	2 (4%)	2.46(0.43–14.2)	0.31
IgG titer; Mean ± SD	6.8 ± 4.7	5.4 ± 2.7		0.09
IL-10 pg/mL; Mean ± SD	7.72 ± 1.88	6.72 ± 1.61		**0.007**

N, number; CI, confidence interval.
Data presented as number (%)].
IL-10, interleukin 10, SD standard deviation.
A bold *P*-value indicates a significant difference between groups (*p* < 00.05).

Table 3

Demographic and lifestyle data of anti-*T. gonii IgG* seropositive and seronegative JIA patients.

Parameter	IgG seropositive JIA N = 17	IgG seronegative JIA N = 26	p
Age, mean $\pm$ SD (years)	10.77 $\pm$ 3.26	10.12 $\pm$ 2.58	0.23
Age at the disease onset (years)	6.3 $\pm$ 2.7	5.6 $\pm$ 2.4	0.38
Gender, N (%)			
Males	3 (17.65%)	10 (38.46%)	0.15
Females	14 (82.35%)	16 (61.54%)	0.15
Residency, N (%)			
Urban	7(41.18%)	8 (30.77%)	0.49
Rural	10 (58.82%)	18 (69.23%)	0.49
Contacts with cats (yes) N (%)	5 (29.41%)	3 (11.54%)	0.90
Eating undercooked meat (yes) N (%)	1 (5.88%)	1 (3.85%)	0.99
Eating raw vegetables (yes) N (%)	2 (11.76%)	1(3.85%)	0.82

Data presented as mean $\pm$ SD, or as the number [%].

N, number; SD, standard deviation; JIA, Juvenile idiopathic arthritis.

Table 4

Demographic and lifestyle data of anti-*Toxocara* species seropositive and seronegative JIA patients.

Parameter	IgG-seropositive JIA N = 4	IgG-seronegative JIA N = 39	p
Age, mean $\pm$ SD (years)	9.10 $\pm$ 2.08	10.48 $\pm$ 2.89	0.38
Age at the disease onset (years)	4.9 $\pm$ 2.5	5.1 $\pm$ 2.6	0.88
Gender N (%)			
Males	1 (25%)	12 (30.77%)	0.81
Females	3 (75%)	27 (69.23%)	0.81
Residency, N (%)			
Urban	1 (25%)	14 (35.9%)	0.67
Rural	3 (75%)	25 (64.10%)	0.67
Contact with dogs (yes) N (%)	1 (25%)	2 (5.13%)	0.14
Contacts with cats (yes) N (%)	1 (25%)	7 (17.95%)	0.73
Eating undercooked meat (yes)	0	2 (5.13%)	0.65
Eating raw vegetables (yes) N (%)	1 (25%)	2 (5.13%)	0.14

Data presented as mean $\pm$ SD, or as the number [%].

N,number; SD, standard deviation; JIA, Juvenile idiopathic arthritis.

and four of them used tocilizumab). In comparison, 40 patients (93%) used nonbiologic DMARDs therapies (35 cases were on methotrexate, two cases were on sulfasalazine, and three cases were on leflunomide). However, 36 cases (83.7%) were on corticosteroid therapy and 18 cases (41.9) were on combined biologic and nonbiologic DMARDs therapies.

JIA patients on glucocorticoid, biologic, DMARDs, and both biologic and nonbiologic DMARDs therapies showed significantly higher seropositivity for anti-*T. gondii* IgG compared to seronegative patients (p = 0.03, 0.009, 0.03, 0.02 respectively) (Fig. 2), however, there were no significant differences between seropositive *Toxocara* JIA patients compared to seronegative in terms of the received therapies (p > 0.05) (Fig. 3).

4. Discussion

The relationship between toxoplasmosis and toxocariasis with rheumatic diseases is still up for debate. There are few studies investigating the relationship between rheumatologic disease and parasitic infection, and to our knowledge, this is the first study conducted to estimate the frequency of *T. gondii* and *Toxocara* among children with JIA and evaluate its relation to the disease activity, IL-10, and the type of received therapies.

The result of the current study showed a significantly high frequency of *T. gondii* infection among children with JIA compared to controls. When the Odds ratio was applied, it revealed that the seropositivity of anti-*Toxoplasma* IgG was 3.43 folds higher among JIA patients compared to controls and this finding was statistically significant. However, the high seroprevalence of *Toxoplasma* in patients with JIA is not related to demographic and socio-economic factors.

There are no previous studies evaluating the frequency of *T. gondii* among JIA patients available to compare this data with. However, *T. gondii* infection is progressively being reported in patients with arthritis and RA in different countries (Fischer et al., 2013; Salman and Mohammed, 2015; El-Sayed et al., 2016; El-Henawy et al., 2017; Hezarjaribi et al., 2021).

IL-10 appears to have an essential role in the initiation and progression of auto-immune diseases such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, and JIA (Moore et al., 2001; Tian et al., 2014; Fathy et al., 2017).

The result of the present study has demonstrated that JIA patients' serum IL-10 levels significantly increased especially among *T. gondii* seropositive JIA. These findings are in line with previous research (Drozdova et al., 2017; Peng et al., 2021).

Table 5

Clinical and laboratory data of anti-*T. gonii* IgG seropositive and seronegative JIA patients.

Parameter	IgG-seropositive N = 17	IgG-seronegative N = 26	P
Subtypes of JIA number (%)			
Persistent oligoarthritis	3 (17.65%)	6 (23.07%)	0.34
Extended oligoarthritis	4 (23.53%)	2 (7.69%)	
Rheumatoid factor-negative polyarthritis	2 (11.76%)	8 (30.77%)	
Rheumatoid factor-positive polyarthritis	2 (11.76%)	1(3.85)	
Systemic JIA (Still's disease)	6 (35.29%)	9 (34.62)	
Duration of the disease, Mean ± SD (years)	7.44 ± 3.57	2.66 ± 0.58	**0.001**
No of joints, Mean ± SD	1.88 ± 0.3	0.92 ± 0.21	**0.02**
Uveitis N (%)	1 (5.88%)	2(7.69%)	0.99
Chorioretinitis N (%)	0	0	
Encephalitis N (%)			
JADAS-27 score, Mean ± SD	8.39 ± 1.64	4.65 ± 2.73	**0.02**
IL-10 pg/mL, Mean ± SD	8.35 ± 1.91	7 ± 1.85	**0.03**
ESR mm/h, Mean ± SD	47.82 ± 16.16	34.54 ± 16.03	**0.01**
Positive ANA, N (%)	7 (41.2%)	7 (27%)	0.34
Positive RF, N (%)	5 (29.4)	6 (23.1%)	0.64
RBCs million/ μL Mean ± SD	4.48 ± 0.58	4.50 ± 0.45	0.06
Hemoglobin (gm/dL), Mean ± SD	10.57 ± 2.06	10.73 ± 1.95	0.79
WBCs /μL, Mean ± SD	7.77 ± 2.03	7.09 ± 2.42	0.34
Platelet count/μL, Mean ± SD	272.02 ± 95.96	290.71 ± 63.74	0.45

JIA, Juvenile idiopathic arthritis; JADAS-27, 27 joint Juvenile Arthritis Disease Activity Score; ESR, Erythrocyte Sedimentation Rate; ANA, anti-nuclear antibodies; RF, rheumatoid factor; RBCs, Red blood cells; WBCs, White blood cells.

Data presented as mean ± SD, or as the number [%].

IL-10, interleukin 10, SD, standard deviation.

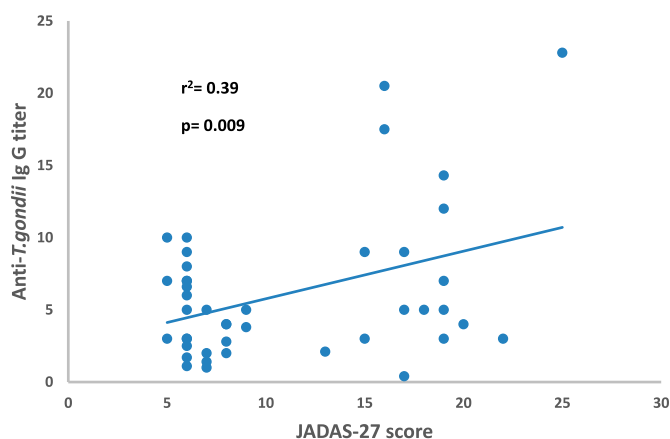A bold P-value indicates a significant difference between groups [$P < 0.05$].

Fig. 1. Correlation between anti-*Toxoplasma gondii* IgG titer, and JADAS-27 score among JIA patients.

IL-10 has been implicated in susceptibility to infection with *T. gondii* and associated with parasite persistence during the chronic phase of toxoplasmosis as it inhibits T-cell and natural killer (NK) cell functions all of which are necessary for pathogen clearance (Couper et al., 2008; Jamal et al., 2021).

Toxocara has been reported among patients with arthritis even among infants and patients with rheumatoid arthritis and anky-losing spondylitis (Williams and Roy, 1981; Van Linthoudt et al., 1990; Jimenez-Balderas et al., 2012).

The present study shows a lack of any significant difference between the JIA patients and controls regarding the seropositivity of *Toxocara* and when the Odds ratio was applied, it revealed that the seropositivity of anti-*Toxocara* IgG was 2.46 folds higher among JIA patients compared to controls and this finding was statistically insignificant. In agreement with our results, Esfandiari et al. (2020) reported that. Anti-*Toxocara* antibodies were not detected in the serum of RA cases. However, Kaplan et al. (2005) demonstrated a significantly higher seroprevalence of *Toxocara canis* in patients with RA compared to the control group.

Juvenile Arthritis Disease Activity Score (JADAS) is a composite disease activity score designed for JIA disease activity in JIA. In patients with rheumatologic disease, few studies reported the association between disease activity and parasitic infection (Consolaro et al., 2009).

The present study has demonstrated a significantly higher JADAS-27 score among JIA children with *T. gondii* seropositive than in seronegative cases and there was a significant positive correlation with anti-*T. gondii* IgG titer and JADAS-27 score. In agreement with

Table 6

Clinical and laboratory data of anti-*Toxocara* species *IgG* seropositive and seronegative JIA patients.

Parameter	IgG-seropositive N = 4	IgG -seronegative N = 39	P
Subtypes of JIA, N (%)			
Persistent oligoarthritis	1(25%)	8 (20.51%)	0.86
Extended oligoarthritis	0	6 (15.38%)	
Rheumatoid factor-negative polyarthritis	1(25%)	9 (23.08%)	
Rheumatoid factor-positive polyarthritis	0	3 (7.69%)	
Systemic JIA (Still's disease)	2 (50%)	13 (33.33%)	
Duration of the disease, Mean ± SD (years)	4.4 ± 2.9	4.3 ± 0.6	0.96
No of joints, Mean ± SD	1.5 ± 0.96	1.28 ± 0.2	0.77
Uveitis, N	1	2	0.26
Chorioretinitis,, N	0	0	
Encephalitis, N	0	0	
JADAS-27 score, Mean ± SD	7.15 ± 1.56	6.02 ± 0.83	0.68
IL-10 pg/mL, Mean ± SD	6.85 ± 1.17	7.6 ± 2.02	0.47
ESR mm/h, Mean ± SD	41.50 ± 13.37	39.62 ± 16.4	0.84
Positive ANA, N (%)	2 (50%)	12 (30.8%)	0.44
Positive RF, N (%)	2 (50%)	9(23.1)	0.25
RBCs million/ μL, Mean ± SD	4.62 ± 0.71	4.64 ± 0.52	0.96
Hemoglobin (gm/dL), Mean ± SD	11.6 ± 2.93	10.57 ± 1.87	0.32
WBCs /μL, Mean ± SD	6.8 ± 2.86	7.42 ± 2.24	0.61
Platelet count/μL Mean ± SD	248.25 ± 66.57	186.92 ± 78.44	0.35

JIA, Juvenile idiopathic arthritis; JADAS-27, 27 joint Juvenile Arthritis Disease Activity Score; ESR, Erythrocyte Sedimentation Rate; ANA, anti-nuclear antibodies; RF, rheumatoid factor; RBCs, Red blood cells; WBCs, White blood cells.
Data presented as mean ± SD, or as the number [%].
IL-10, interleukin 10; SD, standard deviation.
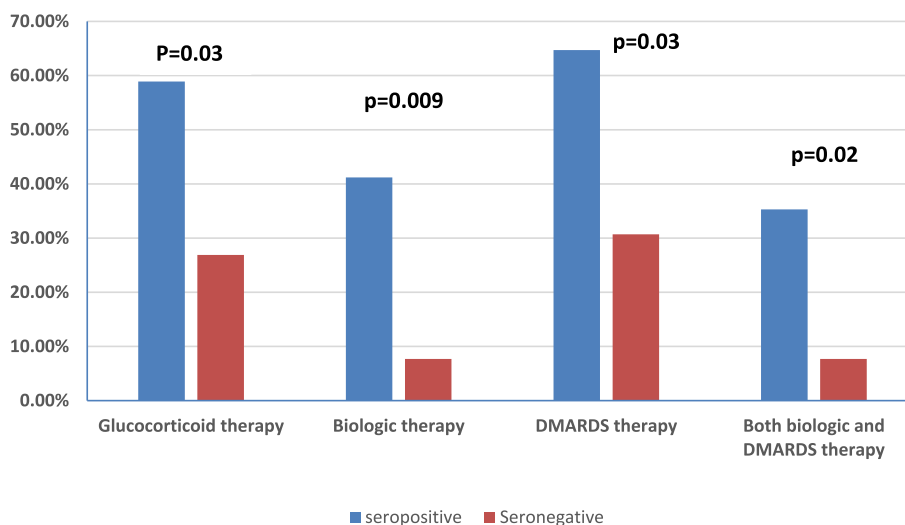A bold P-value indicates a significant difference between groups [$P < 0.05$].

Fig. 2. Relation between the type of therapy and *Toxoplasma gondii* seropositivity among studied cases.

these results, some studies in RA demonstrate a positive correlation between the seropositivity of *T. gondii* and the disease activity markers, especially in high titers (El-Sayed et al., 2016; El-Henawy et al., 2017).

This association can be explained by the binding between the toll-like receptors (TLRs) and *T. gondii* which can cause an inflammatory response that increases the disease activity (Ali et al., 2013).

Among the various clinical subtypes of JIA, seroprevalence rates showed no significant differences between seropositive and seronegative cases. However, the number of affected joints and duration of the disease are significantly higher among *T. gondii* IgG seropositive cases compared to seronegative cases. The increased duration of JIA may create an incline toward more adverse outcomes in the immune system and increase all kinds of infections including *T. gondii*.
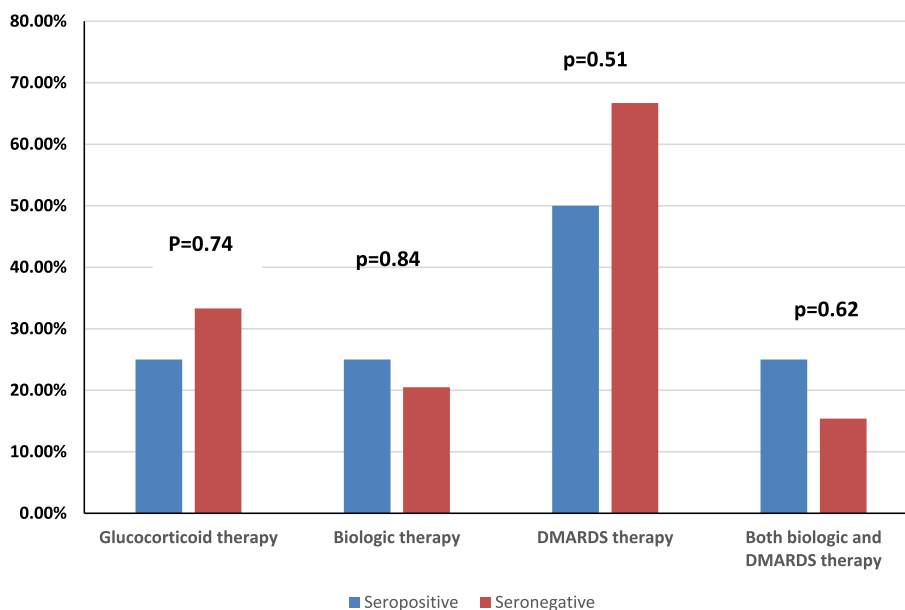
Fig. 3. Relation between the type of therapy and *Toxocara species* seropositivity among studied cases.

JIA patients are exposed to multiple and often combined immunosuppressive drugs that promote the reactivation of a latent *Toxoplasma* infection and may be predisposed to acquire novel infections (İnal and Taş, 2019).

Biological therapy plays a crucial role in improving the outcome of many patients with autoimmune diseases. However, this improvement has been associated with the risk of infection by opportunistic pathogens such as *T. gondii* (Winthrop et al., 2015).

In this work, there was an association between DMARDs therapies and the frequency of *T. gondii* seropositivity in the studied participants. These results are in concordance with the results obtained by other studies (Ellerin et al., 2003; Imperato et al., 2004; El-Sayed et al., 2016).

The biological DMARDs therapy (especially TNF-α inhibitors) in JIA impairs granuloma formations, which are important in limiting the intracellular parasite's growth (Lassoued et al., 2007); Therefore, during the anti-TNF treatment, the risk of *T. gondii* infection should be taken into consideration (Nagy et al., 2019).

It is well acknowledged that non-biologic DMARDs, such as methotrexate (MTX), are an effective JIA treatment. In this study, the patients on none-biologic DMARDs therapy were associated with a high seroprevalence of toxoplasmosis in JIA patients. This result agreed with Kuba et al. (2014) and Etewa et al. (2017) who reported that infected mice treated with methotrexate showed a substantial rise in the seroprevalence of *Toxoplasma* IgM compared to the control group. The high prevalence of seropositivity of *T. gondii* among JIA patients who received MTX may be related to immune system suppression by different mechanisms including antiproliferative effects on B and T cells and this could explain the association between none-biologic DMARDs therapy and *T. gondii* infection (Quéméneur et al., 2003).

In addition, JIA patients on steroid therapy showed significantly higher seropositivity for anti-*T. gondii* IgG. In accordance with our results, Sumyuen et al. (1996) reported that mice that were treated with cortisol acetate for 2 days after the oral acquisition of *T gondii* infection showed a persistent infection, due to immunosuppression. So, it is plausible that glucocorticoid therapy increases the risk of infection, including *T. gondii* (Hoes et al., 2007; Cutolo et al., 2008).

Overall, the findings of the present study support an association between *T. gondii* infection and JIA, suggesting that the high level of IL-10 and immunosuppression caused by the received therapy increases the susceptibility and persistence of *T. gondii* infection or both. In addition, *T. gondii* infection in JIA patients may contribute to the higher disease activity score. Therefore, further larger cohort studies will be necessary to provide further evidence to answer the question of whether *T. gondii* infection contributes to the JIA and disease activity or whether the received therapy in JIA predisposes JIA children to *T. gondii*.

5. Conclusions

The findings of this study support an association between *T. gondii* infection and JIA, disease activity score, and biologic Disease-modifying antirheumatic drugs (DMARDs) therapies. The high level of IL-10 that is associated with JIA increases the susceptibility to *T. gondii* infection and parasite persistence during the chronic phase of toxoplasmosis. As a result, the recommendation for screening tests for *T. gondii* infection among JIA patients is crucial before and during commencing biological therapies, closely monitoring early

signs of infection.

Ethical approval and consent to participate

This case-control study was approved by the Ethics Committee of the Mansoura Faculty of Medicine-Institutional Research Board (approval number R.22.02.1619.).

Consent to participate: All participants' parents gave their informed written consent.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Authors' contributions

Doaa A. Salem: Conceptualization, Methodology, Investigation, Writing – Original Draft Preparation of the work.
Ahmed Hassan, Jameel Alghamdi, Bakheet A. Alghamdi: Methodology and Writing – Review & Editing of the work.
Eman Abdelrazek: Investigation and Writing – Review & Editing of the work.
Amira Ismail: Investigation and Formal Analysis of the work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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