Toxoplasma and Toxocara Seroprevalence in children and adolescents with Juvenile Idiopathic Arthritis and Its relation to disease activity and Type of Therapies: Should We Screen Before Treatment?

Doaa A. Salem (dr_doaasalem@yahoo.com)
Mansoura University
Nanees A. Salem
Mansoura University
Amira Ismail
Mansoura University
Eman Abdelrazek
Mansoura University
Jameel Al ghamdi
Al Baha University

Research Article

Keywords: Toxoplasma gondii, Toxocara, Autoimmunity, Juvenile idiopathic arthritis, Seroprevalence, biological therapies.

Posted Date: March 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1436328/v1

Abstract

Background

In children, juvenile idiopathic arthritis (JIA) is the most frequently encountered autoimmune rheumatic disease. A relationship between *Toxoplasma gondii* and *Toxocara* spp. infections and a variety of autoimmune diseases has been reported. However, there are no studies on the epidemiology and impact of *Toxoplasma* and *Toxocara* infections in juvenile idiopathic arthritis.

Aim

To estimate the seroprevalence of *Toxoplasma gondii* (*T. gondii)* and *Toxocara* in JIA children and adolescents and assess its relation to the disease activity and type of therapy.

Methods

A case-control study was designed to estimate the prevalence and risk factors of *T. gondii* and *Toxocara* infection in 43 JIA patients in comparison to 50 healthy controls using enzyme-linked immunosorbent assay.

Results

There was a significant difference between JIA patients and healthy control subjects as regard anti-T. gondii IgG seroprevalence (P = 0.02) and a non-significant difference in Toxocara seropositivity (P = 0.41). Demographic factors did not affect *T. gondii* and *Toxocara* infection prevalence. Seropositive anti-*T. gondii* IgG cases had a significantly higher disease activity score (JADAS)-27 than seronegative anti-*T. gondii* IgG cases (p = 0.05); with no significant differences regarding anti-*Toxocara* IgG (p > 0.05). The highest *T. gondii* infection seropositivity rate was detected in patients with extended oligoarthritis (4 cases in seropositive vs. two among seronegative, p = 0.34). There was a significant association between immunosuppressive including biological therapies, and *T. gondii* IgG seropositivity (p < 0.05).

Conclusions

The findings of this study support a link between *T. gondii* infection and JIA, disease activity score, and biologic Disease-modifying antirheumatic drugs (DMARDs) therapies. As a result, a recommendation for screening tests for *T. gondii* infection among JIA patients is crucial prior to and during commencing biological therapies and closely monitoring early signs of infection.

Introduction

Autoimmune diseases have been found to be universally on the rise and are now a public health concern as threatening as cancer and heart disease. Juvenile idiopathic arthritis (JIA) is the most prevalent childhood rheumatic disease and the fifth most common chronic illness **(1).**

The etiology is still poorly understood; JIA is a multifactorial condition related to genetic background and environmental factors. The infection has been implicated in the onset and exacerbation of some forms of arthritis in children with known genetic backgrounds **(2).**Many studies were reported a relationship between *Toxoplasma gondii* and *Toxocara* spp. infections and a variety of autoimmune diseases, including rheumatoid arthritis **(3, 4).**

Toxocara canis and *Toxocara catis* are the parasitic zoonosis that causes human toxocariasis. 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 **(5).**

Toxocariasis is acquired by ingestion of the embryonated eggs of the parasite in humans; larvae will travel through a variety of internal organs, causing neurotoxocariasis, ocular toxocariasis, or visceral larva migrans (VLM) **(6).** Larva migrans diagnosis is based on immunological techniques. Enzyme-linked immunosorbent assay (ELISA) using *Toxocara* excretory-secretory (TES) antigens or the use of fractionated TES antigens for Western blotting (TESWB) are two types of tests that are available for the immunodiagnosis of toxocariasis. However, recently the use of recombinant antigens for both serological methods has been demonstrated to be more specific than TES **(7).**

Toxoplasma gondii is an obligate intracellular pathogen that belongs to apicomplexan protozoan **(8).** Nearly one-third of the world's population is infected with this protozoan in developed and developing countries **(9).**

Humans acquired the infection by ingestion of raw or undercooked meat containing tissue cyst or contact with cat feces from the soil and ingesting food or water contaminated with sporulated oocysts **(10)**. Transplacental transmission from infected mothers to the fetus and organ transplants from infected donors are other rare causes of transmission **(11).**

Aim of work:

To estimate the seroprevalence of *Toxoplasma gondii* and *Toxocara species* in JIA patients and how it relates to disease activity and type of treatment.

Subjects And Methods

Study Population and Design

This is a case-control study design conducted on 93 children aged between 5-16 years. The study was carried out between April 2020 and March 2021. Forty-three JIA patients (cases) and 50 apparently

healthy children (controls) age and gender-matched and from the same geographic region were enrolled in the study. The included patients were recruited from the Pediatric Rheumatology Clinic during their routine follow-up visits at Mansoura University Children's Hospital. The inclusion criteria included being over the age of 5, being of any gender, and agreeing to participate in the study voluntarily. JIA patients were recruited regardless of whether they had a recent or previous diagnosis of JIA. The diagnosis and classification of JIA in these cases was based on the International League of Associations for Rheumatology (ILAR) **(12).** Exclusion criteria included age 16 years, diabetes, infection, systemic lupus erythematosus (SLE), malignancy, history or current evidence of infection or comorbidities, and hematologic disease, such as leukemia.

History and clinical assessment :

All participants were subjected to thorough medical history and complete physical examination. Medical history includes demographics, such as age, gender, residence area, history of contact with cats in the home, contact with dogs, and the consumption of raw vegetables and raw/undercooked meat. Clinical data, including 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 **(13).**

Sample Collection and Laboratory Analysis

Blood samples were collected under aseptic conditions and centrifuged at 1000× g for 5 min; serum was collected, stored at −20 ◦C. Serum *T. gondii* IgG antibody was determined by Enzyme-Linked Immunosorbent Assay (ELISA) using commercial kits (Biokit Diagnostics Company, Spain). *T. gondii* IgG ≥10 IU/mL considered as seropositive. Detection of anti-*Toxocara* IgG serum antibodies was performed using enzyme-linked immunosorbent assay (ELISA) kit (NovaTec Immunodiagnostics, Dietzenbach, Germany). Anti-*Toxocara* IgG serum antibodies were quantified, and a cut-off of ≥ 11 IU/mL was used for seropositivity. 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.

Statistical analysis

Data analysis was done using Statistical Package for Social Sciences (SPSS software version-20). The categorical data were presented as number and percent. 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 for skewed distributed variables as median (min-max). The comparisons between two groups were performed with student t-test for parametric data and Mann Whitney test for non-parametric data as appropriate. Additionally, $P < 0.05$ was the statistically significant level.

Results

A total of 93 participants (43 JIA and 50 healthy controls). The controls were combatable with age, gender, and residency of patients (P = 0.17) (P = 0.49) (P = 0.47) respectively. There was no significant difference in lifestyle variables, including contact with cats and dogs, eating of raw or undercooked meat between cases and controls (P□0.05) (Table 1)

Table 1
Demographic data of studied participants

Parameter	Cases 43	Controls 50	p
Age (years)	10.35 ± 2.84	11.07 ± 2.62	0.17
Gender			
Males (number, percent)	13 (30.2%)	14 (28%)	0.49
Females	30 (69.8%)	36 (72%)	
Residence			
Rural	28 (65.1%)	34 (68%)	0.47
Urban	15 (34.9%)	16 (32%)	
Dog contact (yes)	3 (7%)	2 (4%)	0.13
Cat Contact (yes)	8 (18.6%)	3 (6%)	
Eating undercooked meat (yes)	2 (4.7%)	3 (6%)	0.57
Eating raw vegetables (yes)	3 (7%)	3 (6%)	
Data presented as mean ± SD, or as number [%].			

Toxoplasma gondii IgM enzyme-linked immunosorbent (ELISA) assay was negative for all participants.

Anti-*T. gondii* IgG antibodies are significantly more prevalent in children and adolescents with JIA (17/43, 39.5%) in comparison with controls (8/50, 16%) (p = 0.02), however, *Toxocara* IgG antibodies in patients with JIA (4/43, 9.3%) did not differ significantly compared with 2 controls (4%) (P = 0.41) (Table 2).

Table 2
Seroprevalence of *Toxoplasma gondii* and *Toxocara* among studied cases

Parameter	Cases n (%) 43	Controls n (%) 50	Odd ratio (95% CI)	p
Toxoplasma gondii				
Seropositive	17 (39.5%)	8 (16%)	2.47(1.19–5.15)	**0.02**
Toxocara species				
Seropositive	4 (9.3%)	2 (4%)	2.33(0.49–12.11)	0.41
CI, confidence interval				
Data presented as number [%].				
Bold *P*-value indicates significant difference between groups [*P* < 00.05].				

There was no statistically significant association between *T. gondii* seropositive, *Toxocara* seropositive JIA cases, and any of the tested demographic variables (Tables 3 and 4).

Table 3
Toxoplasma gondii seropositivity according to demographic data among studied cases

Parameter	Seropositive 17	Seronegative 26	p
Age (years)	10.77 ± 3.26	10.12 ± 2.58	0.23
Gender			
Males	3 (17.65%)	10 (38.46%)	0.19
Females	14 (82.35%)	16 (61.54%)	
Residence			
Urban	7(41.18%)	8 (30.77%)	0.53
Rural	10 (58.82%)	18 (69.23%)	
Contacts with cats	5 (29.41%)	3 (11.54%)	0.90
Eating undercooked meat	1 (5.88%)	1 (3.85%)	0.99
Eating raw vegetables	2 (11.76%)	1(3.85%)	0.82
Data presented as mean ± SD, or as number [%].			

Table 4
Toxocara species seropositivity according to demographic data among studied cases

Parameter	Seropositive 4	Seronegative 39	p
Age	9.10 ± 2.08	10.48 ± 2.89	0.38
Gender			
Males	1 (25%)	12 (30.77%)	0.81
Females	3 (75%)	27 (5.13%)	
Residence			
Urban	1 (25%)	14 (35.9%)	0.99
Rural	3 (75%)	25 (64.10%)	
Contact with dogs (yes)	1 (25%)	2 (5.13%)	0.28
Contacts with cats (yes)	1 (25%)	7 (17.95%)	
Eating undercooked meat (yes)	0	2 (5.13%)	0.99
Eating raw vegetables (yes)	1 (25%)	2 (5.13%)	0.28
Data presented as mean ± SD, or as number [%].			

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). At the same time, there was no significant difference regarding *T. canis* seropositive cases (p = 0.68) (Table 6). However, there was no evidence of a significant association between JIA cases with seropositive *T. gondii*, *T. canis* seropositive cases with the other studied clinical and laboratory variables (i.e., subtypes of JIA, ANA, RF, CBC, and type of therapy) except for the duration of the disease, number of joints and ESR were significantly higher.

Table 5
Toxoplasma gondii seropositivity according to clinical and laboratory data among studied cases

Parameter	Seropositive 17	Seronegative 26	P
Subtypes of JIA			
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 (years)	7.44 ± 3.57	2.66 ± 0.58	**0.001**
No of joints	1.88 ± 0.3	0.92 ± 0.21	**0.02**
Uveitis	1 (5.88%)	2(7.69%)	0.99
Chorioretinitis	0	0	
Encephalitis	0	0	
JADAS-27	8.39 ± 1.64	4.65 ± 2.73	**0.02**
ESR mm/hour	47.82 ± 16.16	34.54 ± 16.03	**0.01**
ANA (positive/negative)	7/10	7/19	0.26
RF (positive/negative)	5/12	6/20	0.45
RBCs million/ µL	4.48 ± 0.58	4.50 ± 0.45	0.06
Hemoglobin (gm/dL)	10.57 ± 2.06	10.73 ± 1.95	0.79
WBCs /µL	7.77 ± 2.03	7.09 ± 2.42	0.34
Platelet count/µL	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, antinuclear antibodies; RF, rheumatoid factor; RBCs, Red blood cells; WBCs, White blood cells

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

Bold P-value indicates significant difference between groups [$P < 00.05$].

Table 6
Toxocara canis seropositivity according to clinical and laboratory data among studied cases

Parameter	Seropositive 4	Seronegative 39	P
Subtypes of JIA			
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 (years)	4.4 ± 2.9	4.3 ± 0.6	0.96
No of joints	1.5 ± 0.96	1.28 ± 0.2	0.77
Uveitis	1	2	0.26
Chorioretinitis	0	0	
Encephalitis	0	0	
JADAS-27	7.15 ± 1.56	6.02 ± 0.83	0.68
ESR mm/hour	41.50 ± 13.37	39.62 ± 16.4	0.84
ANA (positive/negative)	2/2	12/27	0.56
RF (positive/negative)	2/2	9/30	0.27
RBCs million/ µL	4.62 ± 0.71	4.64 ± 0.52	0.96
Hemoglobin (gm/dL)	11.6 ± 2.93	10.57 ± 1.87	0.32
WBCs /µL	6.8 ± 2.86	7.42 ± 2.24	0.61
Platelet count/µL	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, antinuclear antibodies; RF, rheumatoid factor; RBCs, Red blood cells; WBCs, White blood cells

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

Bold P-value indicates significant difference between groups [$P < 00.05$].

The biological agents given in the study were etanercept 25 mg/week, tocilizumab 200mg/2 week, and infliximab 100 mg/2months. In comparison, the traditional DMARDs were methotrexate 12.5–25 mg /week, sulfasalazine1500-2000 mg /day, and leflunomide 10 mg /every other day.

There was an association between glucocorticoids, DMARDs, and biologic therapy and *T. gondii* IgG seropositivity ($p < 0.05$) but not with *T. canis* IgG seropositivity among the participating cases ($p > 0.05$) (Fig. 1, 2).

Discussion

There is increasing interest in exploring the link between *Toxoplasma* and *Toxocara* infections and autoimmune diseases. Recently, there has been a relationship between *Toxoplasma gondii* and *Toxocara* spp. infections and various autoimmune disorders, including rheumatoid arthritis but a clear relationship between toxoplasmosis and toxocariasis and JIA, has not been well documented. **(3,4) .**

The current study was conducted to estimate the seroprevalence of *Toxocara* and *Toxoplasma* in cases with juvenile idiopathic arthritis, its relation to the disease activity, and emphasize the importance of screening for both prior to immunosuppressive treatment.

The result of the current study showed a high prevalence of *T. gondii* infection among children with JIA (39.5%) compared to controls (16%) ($P < 0.05$) based on IgG seropositivity. However, the difference between cases and controls was statistically not significant as regard *Toxocara* IgG seropositivity ($p = 0.27$).

To our knowledge, there are no studies evaluating the seroprevalence of *T.gondii* in JIA patients are available to compare this data with. However, *Toxoplasma gondii* infection is progressively being reported in patients with arthritis in different Arab countries e.g., Tunisia (58.4%) **(14)**, Iraq (54.0%) **(15)**, and Egypt (54.0%) **(16)** and 76.7% **(17)**, in Iran **(18) ;** and Europe (63.0%) **(19).**

The higher seroprevalence of anti-*T. gondii* IgG antibodies among JIA patients versus control patients which were reported in this work reflect an association between T. gondii infection and JIA.

The pathophysiology behind JIA disease is poorly understood. The significant association between toxoplasmosis and autoimmune diseases may be because *T. gondii* infection may act as a possible cofactor that triggers the development of autoimmune disease by different possible mechanisms **(20).**

T. gondii infection induced exhaustion of cytotoxic T lymphocyte and this will lead to losing their capacity to proliferate, cytokine production leads to the development of of different autoimmune **(21).**

Also, T. gondii infection initial innate immune response led by neutrophils. IL-17 is the major cytokine for neutrophil recruitment after infection and is a potent proinflammatory mediator involved in the pathogenesis of many autoimmune illnesses **(22).**

The increased prevalence of *T. gondii* infection in JIA children and adolescents may be also related to the disturbances in the immune system in JIA that attenuate adaptive cellular immunity, essential for controlling intracellular pathogens such as *T. gondii.* JIA is associated with alterations in the T cell

repertoire, a decline in clonal expansion of naïve T cells in response to a previously unknown antigen, and a decline in newly generated naïve T cells which migrate from the thymus to the periphery (**23**).

JIA children and adolescents are exposed to multiple and often combined immunosuppressive drugs that promoted the reactivation of a latent *Toxoplasma* infection and may be predisposed to acquire novel infections **(24).**

In this work, there was an association between Immunosuppressive therapies and the frequency of *T. gondii* seropositivity in the studied participants. These results are in concordance with the results obtained (**16, 25)** who reported high prevalence of T. gondii among patients with different autoimmune diseases treated by immune-suppressive therapy.

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* **(26).**

The higher risk of *T. gondii* infection in JIA patients receiving biological therapy (especially TNF-α inhibitors) is because TNF-alpha is essential for granuloma formations, which are important in limiting the intracellular parasite's growth (**27)**; Anti-TNF agents used in JIA disease treatment will create an incline towards all kinds of infections, especially granulomatous disorders, including toxoplasmosis **(28).**

This study has demonstrated a significantly higher JADAS-27 among JIA children with *T. gondii* seropositive than seronegative cases (8.39 ± 1.64 vs. 4.65 ± 2.73; p = 0.02).

This association may be due to some toll-like receptors (TLRs) in mammals that had been identified, for which some pathogens act as ligands. Different immune responses can be induced due to binding between the TLRs and pathogens. According to the literature, *T. gondii* may be used as a ligand for TLRs, which can cause an inflammatory response. Therefore, this enhanced inflammatory response can cause increased disease activity **(29).**

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 is significantly higher among *T. gondii* IgG seropositive cases compared to seronegative cases ($P < 0.05$), and this may be related to increasing duration, creating an incline towards more adverse outcomes on the immune system and increase all kind of infections including *T.gondii.*

Conclusions

The findings of this study support a link between T. gondii infection and JIA, disease activity score, and biologic Disease-modifying antirheumatic drugs (DMARDs) therapies. As a result, recommcomindation for screening tests for T. gondii infection among JIA patients is crucial prior to and during commencing biological therapies, closely monitoring early signs of infection.

Declarations

Ethics approval

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

Consent to participate

All participants' parents gave their informed written consent.

Conflict of interest

The authors declare no competing interests.

Funding

The authors did not receive support from any organization for the submitted work.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Contributions

Doaa Salem design of the work; Laboratory work, drafted the work and substantively revised it.

Nanees Salem. Eman Abdelrazek, History Taking, Clinical examination, revised work.

Amira Ismail, Jameel Alghamdi, Data analysis, revised work

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Figures

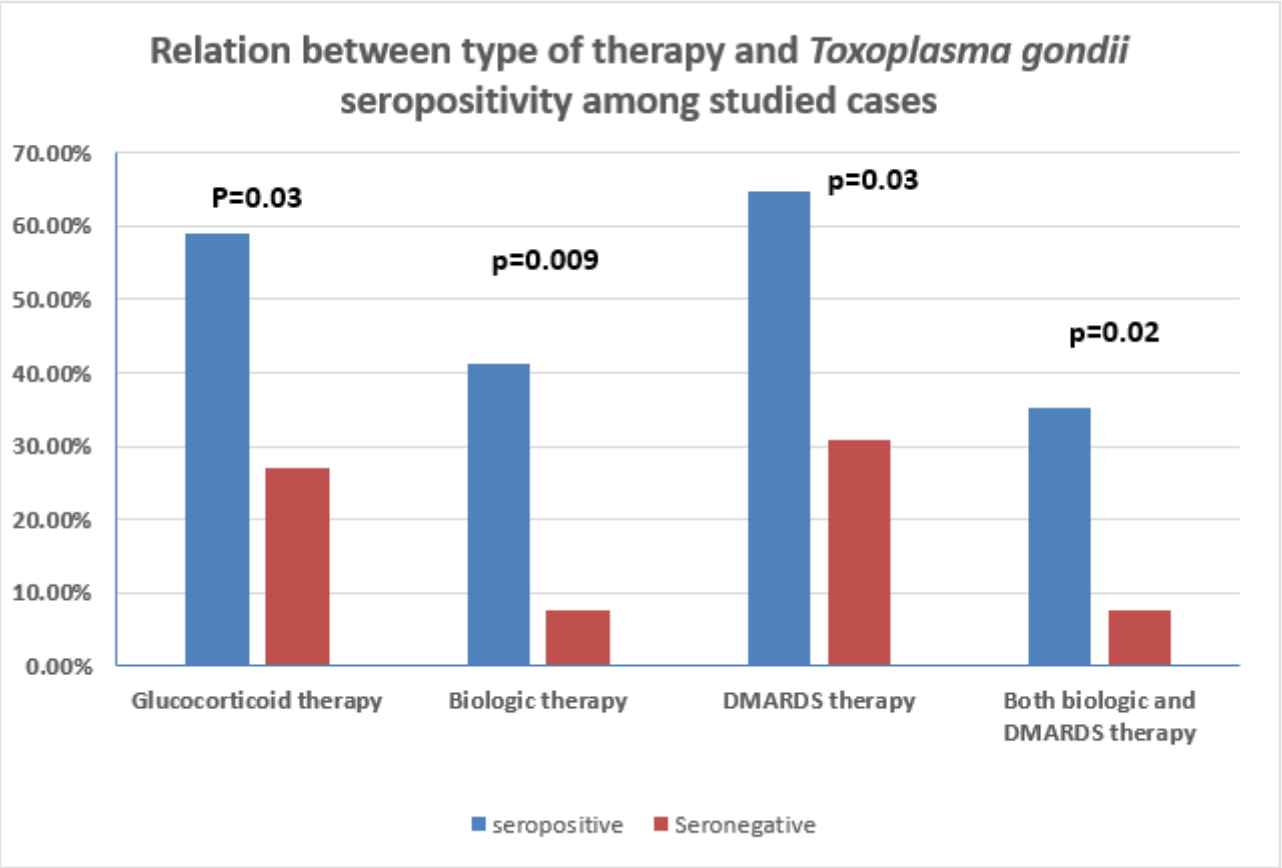

Figure 1

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

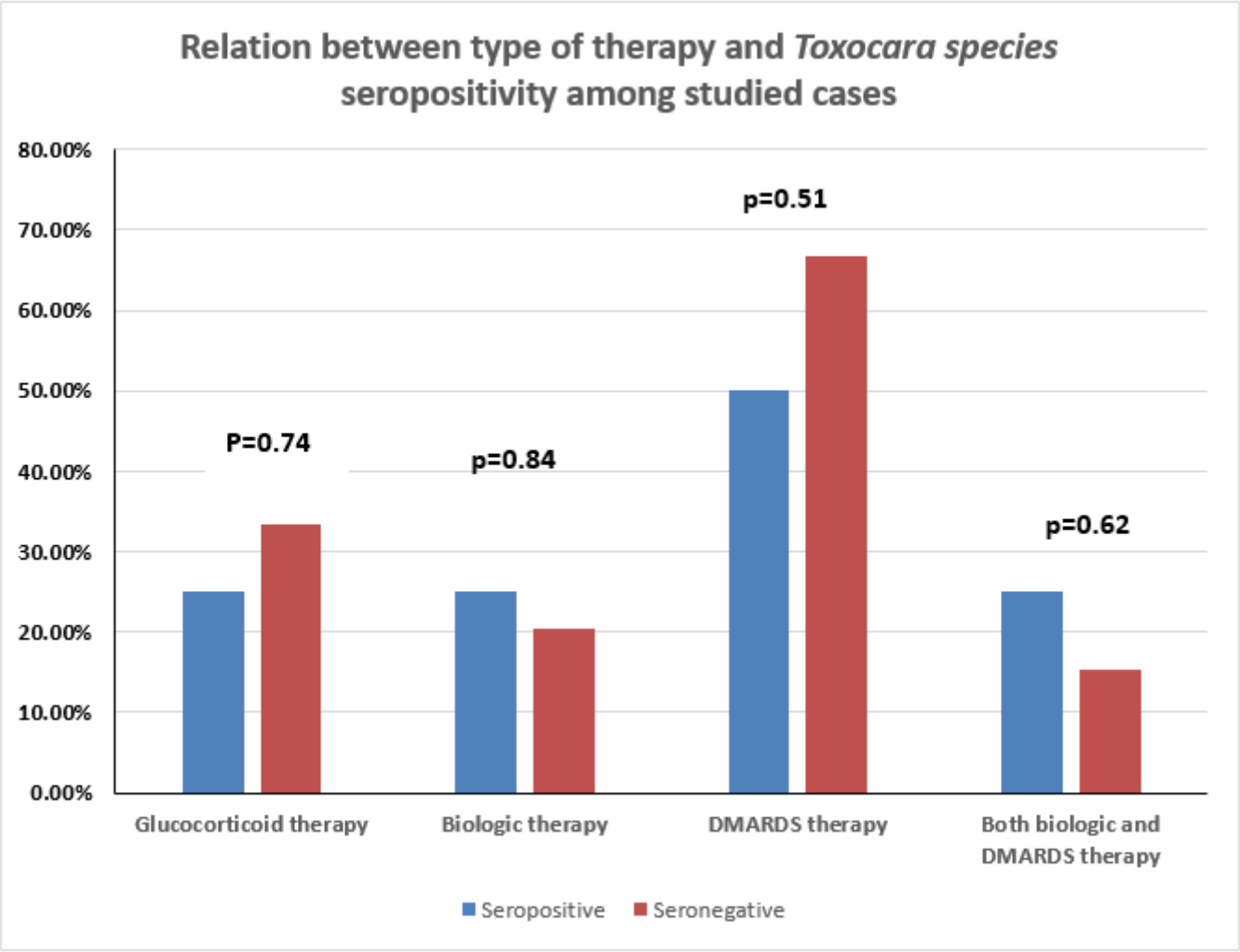

Figure 2

Relation between the type of therapy and *Toxocara species* seropositivity among studied cases