

Comparison of *Giardia lamblia* Infection Status in Asymptomatic and Symptomatic Pediatric Heart Transplant Patients: A Parasitic Infection Assay

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ABSTRACT

Background: Giardiasis is one of the opportunistic infections in immunocompromised patients, especially among organ transplant recipients.

Objective: This study aimed to investigate the prevalence of *Giardia lamblia* infection in children with heart transplantation.

Methods: A prospective cross-sectional study was conducted on 53 heart transplant recipients (aged 1–17 years). Transplant patients were on oral Trimethoprim/Sulfamethoxazole (TMP-SMX) from the first day of transplantation as a prophylaxis regimen. The prevalence of *Giardia lamblia* was evaluated on stool samples by phenotypic assay and polymerase chain reaction (PCR) method.

Results: Out of 53 patients studied, 11 (20.75%) had gastrointestinal symptoms, and 42 (79.25%) were asymptomatic cases. No significant difference was observed between patients with and without gastrointestinal symptoms regarding type of heart disorders ($p=0.13$). The overall prevalence of *Giardia lamblia* infection among heart transplant patients was 5.7% ($n=3$). Moreover, the frequency was different between gastrointestinal symptomatic and asymptomatic recipients (27.27% and 0%, respectively). All three patients whose stool exams were phenotypically positive for *Giardia lamblia* were confirmed with PCR. Out of three, two *Giardia lamblia* isolates were found to have genotype B, while one isolate had genotype A. All of the *Giardia* positive patients suffered from chronic diarrhea and anorexia. *Cryptosporidium* spp., *Isospora belli* and *Blastocysts* spp. were not found in these cases.

Conclusion: The incidence of *Giardia lamblia* infection in pediatric heart transplant patients is considerable and should be noted. A comprehensive guideline for the assessment of *Giardia lamblia* before and after transplantation is suggested.

KEYWORDS: *Giardia lamblia*; Giardiasis; Diarrhea; Heart transplant; Pediatrics

INTRODUCTION

Pediatric heart transplantation is one of the well-known treatments for children with heart failure who cannot be man-

aged by medical therapy [1, 2]. The first pediatric heart transplant was performed in 1967 [3]. According to the International Society of Heart and Lung Transplantation, since the early 1990s, approximately 500–600 pediatric heart transplants have been performed worldwide each year. In addition, more than 12,000 pediatric heart transplantations have been reported by the International Heart and Lung Association [4].

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Although many improvements have been made in heart transplantation in recent years, infection is considered one of the major problems in these patients [5, 6]. Since patients undergoing organ transplantation are treated with immunosuppressive drugs, they may be susceptible to different types of infections. Parasitic infections (PIs) can involve various parts of the heart (e.g., heart valves, myocardium, pericardium, and endocardium). In addition, diagnosis of infections after transplantation may be difficult because the type and severity of signs and symptoms of the infection may be altered or reduced due to the inability of the immune system to respond [7-9]. Therefore, evaluating the PIs in heart transplant patients is very important and will be of great help in the diagnosis and prevention of its consequences.

Giardia lamblia infection has been reported as one of the most common intestinal parasitic infections in Iran. Results of various studies indicated rates between 1.4–39.5% for *Giardia* infection in Iran [10]. Due to the remarkably high infection rate, *Giardia* assay may be necessary for all heart transplant pediatric recipients.

Regarding the importance of this issue, in this study, we aimed to evaluate the frequency of *Giardia lamblia* infection in pediatric heart transplant patients and compare two groups of symptomatic and asymptomatic cases.

MATERIALS AND METHODS

Study Design

This prospective cross-sectional study was performed on 53 pediatric heart transplant recipients aged 1-17 years during a recall in 2018 at Rajaei Cardiovascular, Medical & Research Center. A checklist was provided that included demographic information, including age, gender, type of heart disease leading to heart transplantation, date of heart transplantation, history of any gastrointestinal disorders or symptoms, and type and dose of immunosuppressive drugs. Transplant recipients were on prednisolone, CellCept and tacrolimus as post-transplant immunosuppressive

protocol. A stool exam had been performed for every transplant candidate; however, acid-fast and chromotrope staining were not performed specifically before heart transplantation. In this study, all transplant recipients were on oral administration of TMP-SMX from the first day of transplantation as a prophylaxis regimen to prevent toxoplasmosis.

Fecal Specimen Sampling

The recipients were divided into two groups: recipients with clinical gastrointestinal (GI) symptoms and asymptomatic recipients. The stool samples of patients with GI symptoms (including chronic diarrhea, acute diarrhea, nausea, etc.) and without GI symptoms were collected. The samples were then transferred to the parasitology laboratory of the Research Center of Pediatric Infectious Diseases in Iran University of Medical Sciences.

Furthermore, the stool samples were examined by wet mount examination with Phosphate-buffered saline (PBS) and the formalin ether concentration method. Trichrome staining was also used for more confirmation. The modified acid-fast staining method was performed to detect coccidia [11]. The chromotrope staining was used to detect microsporidia spores as described before [12]. All smear specimens were prepared, stained, and finally observed by a light microscope.

PCR Method

Polymerase chain reaction (PCR) was used to detect the presence of the *Giardia lamblia* parasite. DNA extraction was performed (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The nested PCR was performed using specific primers, and a fragment of the SSU-rDNA (292 bp) gene was amplified [13].

PCR was performed in a 25 µL mixture containing the template (3 µL of DNA), 2.5 U of Taq DNA polymerase, 2.5 µL of 10x PCR buffer, 20 pmol of each primer, 100 µmol dNTPs, and 0.15 mmol MgCl₂.

A total of 35 cycles, with an initial hot start at 96°C for 2 minutes of 96 °C for 45s, 58°C

Table 1: Comparison of frequency between different age groups with and without gastrointestinal symptoms.

Age Groups	Gastrointestinal Signs		P value
	Yes	No	
<5 years	3 (27.27%)	7 (16.66%)	0.71
6-10 years	4 (36.36%)	15 (35.71%)	
11-15 years	4 (36.36%)	17 (40.47%)	
>15 years	0	3 (7.14%)	

for 30 seconds, and 72 °C for 45s, and a final extension step at 72°C for 4 minutes were performed. For the secondary PCR step, the PCR mixture and condition were identical. Positive and negative controls were also used in each set of PCR reaction [13].

PCR product was electrophoresed on 1.5% agarose gel. Finally, the results of microscopic and molecular studies were compared in two groups of patients with and without GI symptoms.

The second round of PCR was performed with inner primers, and PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostic, Mannheim, Germany) according to the manufacturer's instructions. PCR products were used for direct sequencing using the dye termination method and an ABI 3730xl sequencer.

Ethical Consideration

The study was conducted after receiving approval from the ethics review committee of the Iran University of Medical Sciences (IR.IUMS.REC.1397.1367). Informed consent was taken from all patients' parents before enrollment in the study.

Statistical Analysis

Frequency and percentages, mean and standard deviation, were used to describe the data. The Chi-Square test was used to compare categorical parameters. Quantitative data were compared between two groups using the Independent Sample T-test. The data were analyzed using the SPSS software, version 19. In this study, a p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 53 pediatric heart transplant patients (mean age of 9.65 ± 4.35 years) were included in our study; 31 (58.5%) and 22 (41.5%) were male and female, respectively. Among the patients, 11 (20.75%) (eight males and three females) had gastrointestinal symptoms and 42 (79.25%) (23 males and 19 females) were asymptomatic cases. No significant difference was observed between patients with and without gastrointestinal symptoms regarding type of heart disorders ($p=0.13$). Overall, three patients (5.7%) (two males and one female) were microscopically positive for *Giardia lamblia* infection during a fecal examination, and 50 (94.3%) patients were negative.

The mean ages of patients with and without gastrointestinal symptoms were 8.63 ± 4.08 and 9.91 ± 4.43 years, respectively. There was no significant difference in the mean age of patients with and without gastrointestinal symptoms ($p=0.39$). No significant difference was also observed between patients with and without *Giardia lamblia* infection in different age groups ($p=0.71$) (Table 1).

The most common types of underlying heart disorders were dilated cardiomyopathy (DCM) (72.0%) and familial dilated cardiomyopathy (FDCM) (14%). DCM was the most common cardiac disorder in both groups. The prevalences of DCM in the symptomatic and asymptomatic groups were 50% and 77.5%, respectively. In this study, three patients were positive for *Giardia* infection, including two patients (66.7%) with DCM and one (33.3%) with FDCM. There was no significant difference between patients with and without *Giardia lamblia* infection regarding type of heart disorders ($p=0.99$) (Table 2).

Table 2: Frequency of heart problems in all patients studied.

	Transplant Etiology	Frequency	Valid Percent
Valid	DCM	36	72.0%
	DCM + chemotherapy	1	2.0%
	Familial LQT + DCM	1	2.0%
	FDCM	7	14.0%
	Long QT + TFTC	1	2.0%
	RCM + HCM	1	2.0%
	NCLV + CHB	1	2.0%
	RCM	1	2.0%
	Post-surgery Ebstein anomaly	1	2.0%
	Total	50	100.0%
Total		53	

Nausea and anorexia were the most common symptoms in the symptomatic group (72.7%). Vomiting (36.4%), fever (54.5%), and abdominal pain (63.6%) were the most common symptom, respectively. The incidence of acute and chronic diarrhea was 54.5% and 45.5%, respectively. There was no significant difference in the frequency of gastrointestinal symptoms, except for diarrhea, between the positive and negative *Giardia* infected groups. All three *Giardia* positive patients suffered from chronic diarrhea and anorexia. The two groups showed significant difference regarding prevalence of chronic diarrhea ($p=0.026$) (Table 3).

The frequency of *Giardia lamblia* in patients with and without gastrointestinal symptoms is shown in Table 3. There was a significant difference in *Giardia* frequency between patients with gastrointestinal symptoms and those without ($p<0.0001$). The overall prevalence of *Giardia lamblia* infection among heart transplant patients in our study was 5.7%. However, the frequency was different between symptomatic and asymptomatic patients (27.27% and 0%, respectively). Moreover, *Giardia lamblia* infection was confirmed by PCR in three patients who were considered positive for *Giardia lamblia* infection in the parasitology evaluation (Table 4). *Cryptosporidium* spp, *Isospora belli* and *Blastocysts* spp were not found in any cases.

The three positive *Giardia* PCR products were

sequenced and blasted, and the results showed that two were genotype B and one was genotype A.

DISCUSSION

Intestinal parasitic infection (PI) is a problem for patients receiving immunosuppressive drugs, especially organ transplant recipients. *Cryptosporidium* and *Giardia* are among the most common parasitic infections in transplant recipients, especially in endemic areas [14, 15].

Prevalence of *Giardia lamblia* has been reported to be between 20-60% in developing countries in comparison to 2-7% in developed countries [16, 17], so due to the high rate of infections, *Giardia* assay may be necessary for heart transplant pediatric patients in developing countries.

Results of the present study indicated the presence of *Giardia lamblia* infection in symptomatic pediatric heart transplant recipients. However, no other intestinal parasites like *Cryptosporidium* spp., *Isospora belli*, and *Blastocysts* spp. were found. All cases were on oral TMP-SMX from the first day of transplantation as a prophylaxis regimen, which could be the reason for the non-existence of these opportunistic infections. Therefore, eliminating other parasitic infections with this prophylactic regimen may increase the risk of *Giardia* infection. However, this remains to be further

Table 3: Comparison of gastrointestinal symptoms frequency between positive and negative *Giardia lamblia* patients.

Gastrointestinal Symptoms		<i>Giardia lamblia</i>		P value
		Positive	Negative	
Nausea	Yes	2 (66.7%)	6 (75%)	0.78
	No	1 (33.3%)	2 (25%)	
Vomiting	Yes	0	4 (50%)	0.12
	No	3 (100%)	4 (50%)	
Fever	Yes	1 (33.3%)	5 (62.5%)	0.38
	No	2 (66.7%)	3 (37.5%)	
Anorexia	Yes	3 (100%)	5 (62.5%)	0.21
	No	0	3 (37.5%)	
Illness	Yes	2 (66.7%)	3 (37.5%)	0.38
	No	1 (33.3%)	5 (62.5%)	
Abdominal pain	Yes	2 (66.7%)	5 (62.5%)	0.89
	No	1 (33.3%)	3 (37.5%)	
Bloating	Yes	1 (33.3%)	1 (12.5%)	0.42
	No	2 (66.7%)	7 (87.5%)	
Acute diarrhea	Yes	0	6 (75%)	0.026
	No	3 (100%)	2 (25%)	
Chronic diarrhea	Yes	3 (100%)	2 (25%)	0.026
	No	0	6 (75%)	

studied in the future.

Giardia lamblia infection has been reported as one of the most common intestinal parasitic infections in Iranian children [18-20]. In a study, 1100 stool samples were evaluated in children aged 7-14 years in the north of Iran, and the results of this study showed that 367 (33.3%) children had gastrointestinal PIs. In addition, *Blastocystis hominis* (13.5%) and *Giardia lamblia* (10.6%) were the most prevalent parasites [18]. Another study evaluating gastrointestinal PIs in 203 normal children in the south of Iran showed that 25% of children had at least one type of gastrointestinal parasitic infection and the prevalence of *Giardia* was 5.9% as the most common gastrointestinal parasitic infections in the children [19]. In another survey, the frequency of *Giardia lamblia* among 175 children aged 7 to 15 years in Tehran was 9.1% [20].

Giardia lamblia has also been reported as the most common parasitic infection among transplanted patients [21, 22]. It has been reported

in solid organ transplants, including renal, intestinal, and bone marrow recipients [23-25]. Stool samples of 12 children undergoing bone-marrow transplantation were evaluated by Blakey et al., and *Giardia lamblia* was detected as the main parasitic infection responsible for diarrhea [25].

In another study, 43 solid organ recipients (including liver and kidney) were evaluated to determine the causes of diarrhea. Parasitic infections were found in 18 (41.9%) patients with diarrhea, and the most common parasitic infection was related to *Giardia lamblia* (n=9), *Cryptosporidium parvum* (n=7), followed by *Entamoeba histolytica* (n=1) and *Blastocystis hominis* (n=1) [26].

Azami et al. conducted a cross-sectional study to determine the prevalence of intestinal PI in 150 adult renal transplant patients in Iran. Their results showed that 50 (33.3%) patients had PIS. The identified parasites among transplant patients included *Entamoeba coli* (10.6%), *Endolimax nana* (8.7%), *Giardia lamblia* (7.4%), *Blastocystis* spp. (4.7%), *Iodamoeba butschlii*

Table 4: Prevalence of *Giardia lamblia* in patients with and without gastrointestinal symptoms.

	Gastrointestinal Symptoms	Gastrointestinal Signs		P value
		Yes	No	
<i>Giardia lamblia</i>	Positive	3 (27.27%)	0	<0.0001
	Negative	8 (72.72%)	42 (100%)	
Total		11	42	53

(0.7%), *Chilomastix mesnili* (0.7%) , and *Ascaris lumbricoides* (0.7%) [27]. The prevalence of *Giardia lamblia* in this study is consistent with the findings of our study. It seems that *Giardia lamblia* infection is a significant parasitic infection not only in heart transplant recipients but also in other solid organ transplantations.

So far, there has been no study from Iran on the status of *Giardia lamblia* infection in pediatric heart transplant recipients. Regarding the importance of this issue, both microscopic findings and PCR results of *Giardia lamblia* infection after heart transplantation were investigated in this study in pediatric patients.

The results using PCR-based molecular and microscopic methods showed that the frequency of *Giardia lamblia* infection in heart transplant children was 5.7% and in patients with gastrointestinal symptoms was 27.27%. Furthermore, *Giardia*-positive patients had chronic gastrointestinal symptoms such as diarrhea, anorexia, vomiting, and nausea.

While *Giardia Lamblia* may be self-limiting in immunocompetent cases, it has been described by previous studies that chronic diarrhea, anorexia, failure to thrive are the main clinical manifestations of *Giardia Lamblia* in immunodeficient patients [28].

In the present study, results of PCR sequencing demonstrated that out of three isolated *Giardia lamblia*, two were genotype B and one was genotype A; however, in all three *Giardia* positive patients, chronic diarrhea and anorexia were observed.

Assemblages A and B are two distinct genotypes of *Giardia lamblia* that commonly infect humans, and the results of a study indicated that there was no significant difference in the gastrointestinal symptoms between the two types [28]. However, others have reported that children infected with assemblage A were more likely to be symptomatic [29, 30]. The number of *Giardia* positive cases was scarce in our study; therefore, the relationship between genotype and symptoms could not be judged.

Some intestinal parasitic infections like *Cryptosporidium* and *Blastocystis* infections were not found in the present study, probably secondary to a prophylactic regimen of TMP-SMX among heart transplant recipients.

As a limitation of the study, we acknowledge the relatively small number of participants. However, it should be noticed that the number of pediatric heart transplant recipients is limited in Iran.

According to our findings, screening of *Giardia* is recommended in all pediatric heart transplant candidates before and after transplant. The incidence of *Giardia lamblia* infection in gastrointestinal symptomatic heart transplant children is considerable. The necessity of serial evaluation for *Giardia* infection in heart transplant children and a comprehensive guideline for assessment of *Giardia lamblia* before and after transplantation should be considered.

CONFLICTS OF INTEREST: None declared.

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