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Consistency of direct microscopic examination and ELISA in detection of *Giardia* in stool specimen among children

Zohreh Torabi¹, Ali Niksirat¹, Saeideh Mazloomzadeh^{2,3,4}, Akefeh Ahmadiashar^{1,2,3}¹Mousavi Hospital, Zanzan University of Medical Sciences, Zanzan, Iran²Metabolic Disease Research Center, Zanzan University of Medical Sciences, Zanzan, Iran³Social Determinant of Health Research Center, Zanzan University of Medical Sciences, Zanzan, Iran⁴Social Medicine Department, Zanzan University of Medical Sciences, Zanzan, Iran

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ABSTRACT

Objective: To investigate the consistency of direct microscopic examination and ELISA for determination of *Giardia* in stool specimen.**Method:** Study population consisted of children with any clinical symptoms of *Giardia* infestation since last two weeks. Fresh stool specimen was collected from each child. The stools specimens were assessed by two methods of direct microscopic examination and ELISA. The degree of agreement between direct stool exam and ELISA was calculated by Cohen's kappa coefficient.**Results:** In this study, 124 children with age range 2–12 years were investigated. A total of 64 (61.7%) and 79 (65.7%) of children had *Giardia* by direct stool exam and ELISA test respectively. There was association between frequency of constipation and *Giardia* infection ($P=0.036$). The Cohen's kappa coefficient calculated for degree of agreement between direct stool exam and ELISA showed $\kappa=0.756$ ($P<0.001$).**Conclusions:** The frequency of *Giardia* infection in symptomatic children was high and there was high agreement rate between ELISA and direct stool smear.

1. Introduction

Giardia lamblia is a flagellated protozoan that infects the duodenum and small intestine. It is one of the most common intestinal parasites throughout the world^[1–3]. Its higher incidence was reported in childcare centers, and related to poor hygiene and low socioeconomic conditions^[4,5]. Transmission occurs via contaminated water or food. It is also spread by person to person contact^[6,7]. The clinical features of infection might varied from asymptomatic to symptoms of diarrhea, epigastric pain, nausea, vomiting and weight loss^[8]. The prevalence of diarrhea among infants under 12 months was reported higher than control^[9], although, some studies showed the lower incidence of *Giardia* infection in patient with diarrhea^[10,11]. The prevalence of *Giardia* infection in

our country were reported to be about 14% in children and 6% in adults, therefore it should be considered as an important health problem and early detection of infection, appropriate treatment and improvement of health could dramatically reduce the rate of infection^[3,12]. Diagnosis of *Giardia* infestation is routinely done through classical microscopic-based techniques. However, its sensitivity might be reduced because of intermittent shedding of microorganism or presence of organism in low number^[13]. Antigen detection methods such as the direct fluorescent-antibody tests detecting intact organisms and the enzyme immunoassays detecting protozoan soluble antigens in stool and ELISA are widely used. In addition to higher sensitivity and specificity, a large number of faecal specimens can be screened at one time by these methods with less technical skills, less costs, less labour, and less laboratory turnaround times^[13,14]. However, some studies showed lower specificity or sensitivity of immunoassay tests^[15,16]. In this study the correlation of symptoms of patients with *Giardia* and positive direct stool smear was investigated. All samples were also examined by ELISA test and agreement between results of ELISA and direct stool smear were measured.

*Corresponding author: Akefeh Ahmadiashar, Sub specialist of Immunology and Allergy, Associate Professor of Pediatrics, Pediatric Ward, Metabolic Disease Research Center and Social Determinant of Health Research Center, Zanzan University of Medical Sciences, Zanzan, Iran.

Tel: +98-241-4131429, +98-9122418830

Fax: 0098-241-7270751

E-mail: akefeh45@zums.ac.ir, zu_afshar@yahoo.com

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2. Materials and methods

This cross sectional study was done from February 2010 to June 2012 in Pediatric Clinic of Mousavi Hospital (Zanjan, Iran). Study population consisted of 2–12 years old children with suspicious clinical symptoms of *Giardia* infestation: at least two symptoms of frequent abdominal pain, diarrhea, flatulence, malaise, anorexia, nausea and weight loss since the last 2 weeks. Children have symptoms of acute gastroenteritis with dehydration, symptoms of upper respiratory tract infection, systemic disease or positive stool smear for other organism or protozoa were excluded from this study. After getting written consent from parents, the patients were consecutively enrolled to this study. Three fresh stool specimens were obtained from each child. The stool samples were collected into a disposable plastic container and then delivered immediately to the laboratory for examination. Fresh fecal samples were analyzed for the presence of cysts or trophozoite in the feces by microscopic examination, using the sucrose gradient concentration method. After staining with Lugol's solution, samples were also examined by ELISA test (Dahlewitz, Berlin, Germany). The age, gender, living place and symptoms of subjects were also recorded. Data were analyzed using the Statistical Package for the Social Sciences software program. The *Chi*-square test was used for assessment of relation of *Giardia* infestation and clinical symptoms. *P* value less than 0.05 was considered statistically significant. Statistical kappa agreement rate was used for direct stool examination and ELISA test agreement rate. A kappa value of 1 indicates perfect agreement and zero shows that the agreement is not better than chance.

3. Results

One hundred and twenty four patients (43 girls and 81 boys), with ages between 2–12 years, were consecutively recruited into the study. A total of 75 persons (60.5%) were living in city and 49 persons (39.5%) in rural areas. Sixty four patients (51.6%) had positive results for direct stool exam of *Giardia*. We didn't find any association between gender, age and living place and infection (Table 1).

Table 1

Characteristics of patients with *Giardia* and without *Giardia* in direct stool examination.

Characteristics	<i>n</i> (%)	<i>Giardia</i> in direct stool smear		<i>P</i> value
		Yes <i>n</i> (%)	No <i>n</i> (%)	
Gender	Girls	43 (34.7)	24 (37.5)	0.490
	Boys	81 (65.3)	41 (68.3)	
Age (years)	2–5	92 (74.2)	47 (73.4)	0.900
	6–9	18 (14.5)	9 (15.0)	
	10–12	15 (11.3)	8 (12.5)	
Living place	City	75 (60.5)	43 (67.2)	0.115
	Village	49 (39.5)	21 (32.8)	

We found significant association between constipation and positive direct stool smear for *Giardia* (21.9% infected *v.s.* 8.3% without infection) (*P*=0.036). However there was no significant relation between *Giardia* infection and symptoms of abdominal pain, diarrhea, nausea, flatulence,

anorexia and weight loss (Table 2).

Table 2

Frequency of symptoms in children and association of them with *Giardia* infection.

Symptoms		<i>n</i> (%)	Positive direct smear	Negative direct smear	<i>P</i> value
			<i>n</i> (%)	<i>n</i> (%)	
Diarrhea	Yes	10 (8.1)	6 (9.4)	4 (6.7)	0.580
	No	114 (91.9)	58 (90.6)	56 (93.3)	
Abdominal pain	Yes	62 (50.0)	34 (53.1)	32 (53.3)	0.472
	No	62 (50.0)	30 (46.9)	28 (46.7)	
Flatulence/ distention	Yes	8 (6.5)	4 (6.2)	4 (6.7)	0.925
	No	116 (93.5)	60 (93.8)	56 (93.3)	
Nausea	Yes	24 (19.4)	12 (18.8)	12 (20.0)	0.860
	No	100 (80.6)	52 (81.2)	48 (80.0)	
Anorexia	Yes	45 (36.7)	21 (32.8)	24 (40.0)	0.406
	No	79 (63.3)	43 (67.2)	36 (60.0)	
Constipation	Yes	18 (15.3)	14 (21.9)	5 (8.3)	0.036*
	No	103 (84.7)	50 (78.1)	55 (91.7)	
Weight loss	Yes	59 (47.6)	26 (40.6)	33 (55.0)	0.109
	No	65 (52.4)	38 (59.4)	27 (45.0)	

*: *P*<0.05 or significant association.

Seventy nine (63.7%) children were positive for ELISA test for *Giardia* infection (Table 3). The degree of agreement between direct stool exam and ELISA was calculated by Cohen's kappa coefficient, $\kappa=0.756$, *P*<0.001.

Table 3

Frequency of *Giardia* detection by microscopic stool exam and ELISA test.

ELISA	Direct stool exam		Total (%)
	Positive <i>n</i> (%)	Negative <i>n</i> (%)	
Positive <i>n</i> (%)	64 (51.6)	15 (12.1)	79 (63.7)
Negative <i>n</i> (%)	0 (0.0)	45 (36.3)	45 (36.3)
Total <i>n</i> (%)	64 (51.6)	60 (48.4)	124 (100.0)

4. Discussion

This study demonstrated higher prevalence of *Giardia* infestation in our patients. It was compatible with study of Al-Saeed and Issa[17]. Therefore attention to possibility of *Giardia* infection in cases with any gastrointestinal discomfort in endemic area and appropriate treatment could be desirable.

We didn't find any association between gender, age and living place and infection. However, One study in South East of Asia demonstrated that *Giardia* was slightly higher in males than in females with age range of 2–5 years old children[18]. Several studies showed higher incidence of *Giardia* infection in rural areas and higher prevalence were reported in older children[3,12,18–20]. The frequency of diarrhea in our study population was very low and its prevalence in patients with positive and negative *Giardia* infection was similar. Abdominal pain was the most prominent symptom, however it didn't show any significant difference in infected and non infected children. Our results was approximately identical with study of Younas *et al*[4]. Some studies implicated the lower frequency of diarrhea in infected patients in comparison to control and even protective role of *Giardia* in lowering the acute diarrhea among school aged children[10,11,20].

In contrast, several studies showed the significant association and high frequency of diarrhea in patients with

Giardia infection, especially in younger ages^[9,21,22].

We found significant association between constipation and *Giardia* infection. In one study by Morch *et al.*, constipation was reported as one of gastrointestinal discomforts in patients after waterborne outbreak of *Giardia* infection^[23].

All of smear positive results had been confirmed through ELISA test. There was also higher number of *Giardia* infection by ELISA test in comparison to direct stool exam. In constant to our study Kocak Tufan *et al.* found that the detection of *Giardia* by ELISA in patients with diarrhea was more precise than direct stool exam^[24]. Another study demonstrated higher sensitivity and lower specificity of immunoassay tests in comparison to microscopic test^[16], and one animal study by Wilson and Hankenson showed lower sensitivity and positive predictive value for rapid ELISA^[15].

This study showed high frequency of *Giardia* infection in patient with gastrointestinal problems and significant association of constipation and *Giardia* infection. Due to the periodic fecal excretion of parasite, frequent stool collection is required and experts are needed for microscopic examination, which are time wasting, ELISA test could be a preferred and cost-effective method for rapid determination of *Giardia* infection considering its higher agreement rate with microscopic examination in our study, in spite of higher specificity of direct stool exam.

Conflict of interest statement

We declare that we have no conflict of interest.

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