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Copro-Antigen versus Classical Microscopy as Diagnostic Tool for *Giardia Lamblia* Infection in Egyptian Patients

Samir A. Shahat[1], Ahmed M. Sallam[1], Hesham M. Gad[2], Mohammed H. Abdallah[1]

[1] Department of Parasitology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

[2] Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

ABSTRACT

Background: *Giardia lamblia*, a flagellate protozoa, is a one of the most common causes of non-viral (parasitic) diarrheal illness in humans. Laboratory diagnosis mainly consists of direct microscopic examination of stool specimen for trophozoites and cysts. However, due to intermittent fecal excretion of the parasite, the patient may be misdiagnosed, continue excreting the parasite and infecting others. Therefore, other methods of diagnosis should be looked for, which overcome the drawbacks of microscopy when used alone for diagnosis. The present study aimed to evaluate the efficacy of copro-antigen detection by ELISA test in comparison to direct microscopy in the diagnosis of *G. lamblia* in stool specimens from patients with diarrhea and other gastrointestinal symptoms.

Patients and methods: stool samples were collected form 250 child included in the present study (150 symptomatic and 100 apparently healthy as a control group) aged between 1-10 years old, and subjected for direct microscopic examination and ELISA test for copro-antigen detection.

Results: out of 250 stool samples, 53 specimens (21.2%) were positive for *Giardia* by direct microscopy, while 68 specimens (27.2%) were positive by ELISA test.

Conclusion: ELISA test for copro-antigen detection in stool samples is a rapid and effective method with high sensitivity and specificity for diagnosis of giardiasis in stool specimens even when the parasitic count is low, thus reducing the chances of missing even in the asymptomatic cases.

Keywords: Direct microscopy, *Giardia lamblia*, RIDASCREEN *Giardia* (ELISA) test.

INTRODUCTION

Giardia lamblia (also known as *Giardia intestinalis*, *Giardia duodenalis* and *Lamblia intestinalis*), a flagellate protozoa, is one of the most common pathogenic gastrointestinal parasites infecting humans [1]. It has a global distribution affecting about 280 million cases yearly (mainly children aged between 1-3 years old) in both developing and developed countries [2]. In recognition of the burden of disease caused by the parasite, the WHO has included giardiasis in the list of neglected diseases since 2004 [3].

Cysts are transmitted through the fecal–oral route due to consumption of contaminated food or water [4].

Clinical manifestations of giardiasis usually appear 1-2 weeks after infection and may range from asymptomatic carrier state to acute fulminating diarrhea or chronic persistent diarrhea, abdominal pain, vomiting, malabsorption and weight loss [5, 6]. It may cause chronic post-infectious complications, including irritable bowel syndrome, via mechanisms that remain obscure [7, 8].

Diagnosis of giardiasis is frequently based on detection of trophozoites or cysts by microscopic examination of stool samples. Multiple, successive fecal samples should be taken and examined over a period of 1-2 weeks because of the intermittent nature of cyst excretion [9, 10]. Special techniques like concentration by formalin ethyl acetate centrifugation or by zinc sulfate floatation and special staining like trichrome staining can also be used in conjugation with microscopy. The main drawback of this method is that it is time consuming and depends on the skill of an experienced laboratory personnel. Also, the parasite may be hidden by bile pigments and not visualized [11].

Immunological diagnosis can also be done based on detection of anti-*Giardia* antibodies in the serum, but cross reaction and poor correlation between positive anti-*Giardia* antibody titers and the presence of active *Giardia* infection can occur [12]. This problem has been overcome by direct detection of *G. lamblia* antigen in stool (Copro-antigen diagnosis) [13]. Various enzyme-linked immunoassays (including ELISAs) have been used and have achieved specificities of (87-100%) and

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sensitivities of (63-100%) [14]. In addition, immuno-chromography (IC) tests have also found achieving specificities of (79–100%) and sensitivities of (26-100%), depending on the study [15].

Advantages that copro-antigen detection methods offer over microscopy are that they have the capacity to detect prepatent infections prior to the excretion of cysts in host feces and can be employed for the cost-effective, sensitive and rapid screening of large numbers of fecal samples. However, like other immuno- or sero-diagnostic techniques, they do not allow the identification of the species or genotypes [16, 17].

Hence, the present study was done to evaluate the efficacy of an FDA approved commercial ELISA kit, RIDASCREEN *Giardia* test (R-Biopharm AG, Darmstadt, Germany) for diagnosing intestinal giardiasis and to compare it with direct microscopic examination of stool samples of patients with gastrointestinal symptoms.

PATIENTS AND METHODS

A group of 150 children were included in the present study; from those attending outpatient clinics of pediatrics at Al-Hussein and Said Jalal University Hospitals, Faculty of Medicine, Al-Azhar University, Cairo, Egypt, aged between 1 to 10 years old, complaining of gastrointestinal symptoms as abdominal pain, vomiting, diarrhea, indigestion, distension, dehydration and weight loss (case group). Also, another group of 100 asymptomatic (apparently healthy) children was selected as a control group. The study was conducted over a period of 6 months, from December 2015 to May 2016. Stool samples were collected from every child and a written informed consent was taken from the child parents before collection of samples.

Stool examination:

Stool samples were collected in a 25 ml clean, dry wide-mouthed plastic containers. Gross examination of the stool samples was performed for color, consistency, mucous, blood and adult parasites. Each sample was divided into 2 parts; the 1st part was used to prepare slides for direct wet smear examination according to **Baroody** [18] and Formalin-Ethyl Acetate sedimentation concentration method according to **Garcia** [19] while the 2nd part was immediately stored at -20º C for copro-antigen detection.

Copro-antigen detection by ELISA:

It was performed using a RIDASCREEN *Giardia* kit (R-Biopharm AG, Darmstadt, Germany). The test was done according to the manufacturer's instructions.

Statistical analysis was performed using direct microscopy as the gold diagnostic standard. The RIDASCREEN *Giardia* ELISA kit was evaluated for sensitivity, specificity and positive predictive value using GraphPad InStat 3 software program.

The study was approved by the Ethics Board of Al-Azhar University.

RESULTS

Out of the 150 symptomatic children (case group); 47 of them were positive for giardiasis using direct microscopy, while 54 children were positive by ELISA test. Among the 100 child representing the control group; 6 children were positive by direct microscopy, while 14 children were positive by ELISA test.

DISCUSSION

Nowadays, antigen detection assays for *G. lamblia* have proven to be very useful with the advantages of reduced labor and time consumption required in its diagnosis. ELISA with sensitivity of 95-100% and specificity over 90% when compared with direct microscopy provides a relevant alternative method with the advantage of increased sensitivity required to confirm infections in patients with low parasite numbers and diagnose the disease even if the live parasite is absent in the fecal samples. RIDASCREEN *Giardia* test is a recent FDA approved enzyme linked immunosorbent assay which detects *Giardia* antigen in stool samples [20, 21].

In the present study, the prevalence of *Giardia* was 21.2% by direct microscopy (53 out of 250 cases) and as high as 27.2% by ELISA (68 out of 250 cases). This is comparable to **Noor *et al.*** [22] and **Singhal *et al.*** [23] studies where the prevalence rates of *Giardia* by direct microscopy were 15.5% and 17.3% respectively and by ELISA were 22.6% and 23.6% respectively. The results may be different due to the different study area, sample size, age group, etc...

In the present work, the sensitivity and specificity of ELISA test in comparison with direct microscopy reached 100% and 92.4% respectively. This is comparable to **Chakarova [24], Noor *et al.* [22]** and **Singhal *et al.* [23]** studies where the sensitivity of ELISA was 98.8%, 100% and 96.8% respectively, and the specificity reached 100%, 91.5% and 91.6% respectively.

In another study, sensitivity and specificity of ELISA test was found to be 76.4% and 100% respectively [12]. This reduced sensitivity can be due to less number of samples included in their study, as the sensitivity of ELISA test has been found to improve with increase in the number of specimens [25].

This means that it is a very good sensitive diagnostic test at finding the disease. However, lower specificity may be due to some cross-reactions with other intestinal parasites or past infection with giardiasis. If ELISA result is negative, it can be fairly said that the patient does not have giardiasis.

CONCLUSION

Although, direct microscopy is considered as gold standard for diagnosis of giardiasis, it may give false negative results, especially, in chronic infection due to intermittent shedding of cysts.

RIDASCREEN *Giardia* (ELISA) test is considered as a rapid and effective method with high sensitivity and specificity in detecting *Giardia* antigens in stool specimens even when the parasitic count is low, thus reducing the chances of missing even in the asymptomatic cases. It is easier to perform and is useful for rapid investigation of large number of stool specimens.

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Table 1: Comparison between results of direct microscopy and ELISA

	Case group		Control group		P-value
	Positive	Negative	Positive	Negative	
Direct microscopy	47	103	6	94	< 0.0001 (s)
ELISA	54	96	14	86	0.0001 (s)
P-value	0.4636 (ns)		0.0970 (ns)		

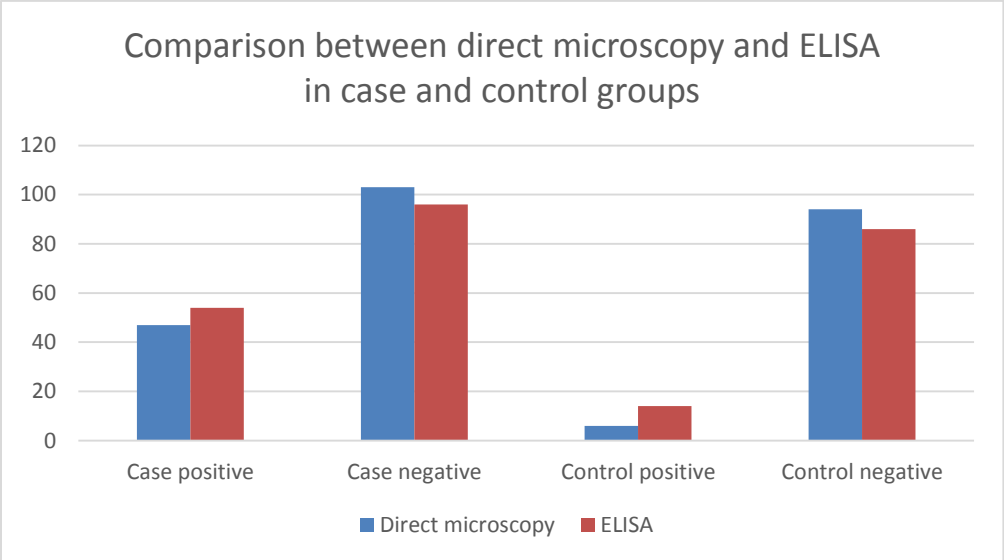

Figure 1: Comparison between results of direct microscopy and ELISA