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Original Article

A comparison of different methods used for Diagnosis of Giardia lamblia in Children Fecal Specimens

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Abstract

Background: Giardia lamblia, a flagellate protozoon, is a common causative agent of parasitic diarrheal diseases of humans. Laboratory diagnosis mainly consists of direct microscopic examination of stool specimen for trophozoite and cysts of Giardia. However, due to intermittent fecal excretion of parasite, the case may be miss diagnosed and the patient may continue excreting the parasite and infecting others. Therefore, other methods of diagnosis are needed, which should overcome the above drawbacks of microscopy used alone.

Objectives: The present study was done to evaluate the efficacy of immunoassay by RIDASCREEN Giardia and Immunochromatographic tests in comparison to direct microscopy in the diagnosis of Giardia lamblia in stool specimens from patients with diarrhea and other gastrointestinal symptoms.

Materials and Methods: At the Children Hospital, Faculty of Medicine, Assiut University, Assiut, Egypt, a total of 200 patients were included in this cross-sectional study and each fecal specimen was taken from each patient which was divided into two parts. One part was used for direct wet mount examination and second part was used for the quantitative and qualitative EIA RIDASCREEN Giardia and Immunochromatographic immunoassays, respectively.

Results: Out of 200 stool samples, 60 specimens (30%) were found to be positive for Giardia lamblia by immunoassay that was significantly better than the conventional direct wet mount microscopy (20% detection). Maximum cases were detected by RIDASCREEN Giardia test with a sensitivity of 100% and a specificity of 91.5%.

Conclusion: RIDASCREEN Giardia test is a rapid and effective method with high sensitivity and specificity and detects Giardia antigens in stool specimens even when the count of parasite is low, thus reducing the chances of missing even the asymptomatic cases.

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Key Words: Direct wet mount microscopy, Giardia lamblia, RIDASCREEN Giardia test, Immunochromatographic test, Stool concentration, Diagnosis.

Introduction

Giardiasis is caused by the protozoan parasite Giardia lamblia (also known as G. intestinalis or G. duodenalis). Giardiasis is considered the most common protozoal infection in humans; it occurs frequently in both developing and industrialized countries. Giardia lamblia, a flagellate protozoan, is a common causative agent of parasitic diarrheal diseases of humans. Giardia as an intestinal flagellate protozoan exists in two stages: An active trophozoite stage and the dormant cyst stage, which is the
infective stage\(^{(1)}\). Transmission of the cyst and the disease occurs mainly by the feco-oral route where humans can be infected by swallowing the Giardia cyst found in contaminated water or food\(^{(2,5)}\).

It is a cosmopolitan parasite with an overall prevalence rate of 20-30% in developing countries. Higher numbers of infections are seen in the late summer months. Travelers to regions of Africa, Asia, and Latin America where clean water supplies are scarce are at increased risk of contracting the infection\(^{(2)}\). Some healthy people do not get sick from *Giardia lamblia*; however, they can still pass the infection on to others. Anyone may become infected with Giardia. However, those at greatest risk are: People in child care settings, those who are in close contact with someone who has the disease, people who swallow contaminated drinking water, backpackers or campers who drink untreated water from lakes or rivers and people who have contact with infected animals\(^{(4)}\). Children, seniors, and people with long-term illnesses may be more prone to contract the infection as the risk of transmission is higher in day care centers and in hypogammaglobulinemia patients; which makes it an opportunistic infection\(^{(5)}\). Clinical manifestations are usually diarrhea, abdominal cramps, nausea, bloating and loss of appetite. In chronic and complicated cases, cholecystitis and malabsorption may be observed\(^{(6)}\). Giardia infection may cause failure to absorb fat and vitamins, and can lead to weight loss. Though, some infections with *Giardia lamblia* have no symptoms\(^{(7)}\).

The most common way of laboratory diagnosis of giardiasis is based on microscopic detection of the trophozoite and cysts in stool samples. The visualization of the cysts or trophozoites in stool specimens usually is time and labor intensive and depends on the skill of an experienced professional\(^{(6)}\). The excretion of Giardia cyst in the stools of patients may be intermittent. For that, in some cases miss diagnosis occurs and the patient may act as a source for infection\(^{(8)}\). Therefore, other ways of diagnosis should be looked for, which can overcome the above drawbacks. Given these difficulties the development of sensitive, cost-effective and rapid diagnostic methods is of utmost importance. Another method of diagnosis that is commonly used as a screening tool is antigen detection assay of stool samples. This method detects a specific protein found in the wall of *Giardia* cysts\(^{(9)}\). Molecular techniques may be resorted for diagnosis in refractory cases\(^{(10)}\).

The present study was done to evaluate the efficacy of immunochromatographic examination and the enzyme immunoassay (EIA) tests for detection of *G. lamblia* soluble copro-antigens in comparison to diagnosis using direct microscopy in stool specimens from patients with diarrhea and other gastrointestinal symptoms.

**Materials and Methods**

**Sample Collection:** 200 stool samples were collected during the period from January to August 2015. Samples were collected from children attending the outpatient clinics of Children Hospital, Faculty of Medicine, Assiut University, Assiut, Egypt, with complaints of diarrhea and other gastrointestinal symptoms. An oral informed consent was taken from custodian of each patient before collection of specimen and patients were anonymously reported after the study approved by the local ethical committee of the faculty. Fresh stool samples were collected in dry, clean, leak-proof plastic disposable cups dated and labeled with name, age, and gender of the patient. Each sample was divided into 2 parts. First part was used to prepare slides for direct wet mount examination and slides prepared after concentration methods. Second part was immediately stored at -20 °C for performing EIA and immunochromatographic tests later.
Sample Examination: Samples were transported to parasitology laboratory, Department of Medical Parasitology, Faculty of Medicine, Assiut University. All stool samples were examined by: 1) Macroscopic examination: It was performed to detect blood and mucus. Stool consistency (i.e., formed, soft, loose or watery) was reported. Steatorrhea was recorded (if present). Adult worms, segments of tape worms, larvae were reported (if present). 2) Microscopic examination: It was performed both directly and after concentration.

Direct smear: Saline, iodine and LPCB (Lacto-Phenol Cotton Blue) wet mounts were done in 3 standard approaches; a) Saline wet mounts were prepared by mixing a small amount of stool (about 2 mg) using an applicator sticks with a drop of physiological saline on a clean glass microscope slide. A cover slip was placed over the mixture. The slide was examined microscopically at 10x and 40x\(^2\), b) Iodine wet mounts were prepared by adding a small volume of stool (about 2 mg) using an applicator sticks to a drop of Lugol’s iodine (diluted 1:5 with distilled water) on a clean glass microscope slide. A cover slip was placed over the mixture\(^2\), and, c) LPCB wet mounts were prepared by mixing a drop of LPCB stain with a small volume of stool (about 2 mg) on a clean glass microscope slide. A cover slip was placed over the mixture. LPCB consisted of 20 g of phenol crystals, 20 mL of lactic acid, 40 mL of glycerol, 0.05 g of cotton blue stain, and 20 mL of distilled water\(^2\).

Concentration technique (formol-ether concentration technique): Using an applicator stick, about 1 g of stool sample was placed in a clean 15 mL conical centrifuge tube containing 7 mL formalin. The sample was suspended and mixed thoroughly with applicator stick. The resulting suspension was filtered through a cotton gauze sieve into a beaker and the filtrate was poured back into conical centrifuge tube. The debris trapped on the sieve was discarded. After adding 3 mL of diethyl ether to the mixture and hand shaken, the content was centrifuged at 2000 rpm for 3 minutes and the supernatant was discarded. Saline, iodine and LPCB wet mounts preparation were examined from the sediments\(^3\).

Immunochromatographic detection of *G. lamblia* soluble copro-antigen: This stool examination was done using rapid solid phase qualitative immunochromatography kit employing monoclonal antibodies (Epitope Diagnostics, Inc. San Diego, CA92126, USA)\(^4\). The *Giardia* copro-antigen test employs dye-conjugated monoclonal antibody against *G. lamblia* antigen and solid-phase/membrane coated specific anti-*G. lamblia* monoclonal antibody. In this test, the specimen was first treated with an extraction solution to extract *G. lamblia* antigens from the feces. Following extraction, the only step required is to screw the *G. lamblia* test strip tube into the sample collection tube. As the sample extraction flows upward through chamber and reach the test strip, the colored particles migrate. In the case of a positive result the specific antibody present on the membrane will capture the colored particles red.

ELISA Ridascreen Giardia test: The enzyme immunoassay test was performed according to manufacturer’s instructions (R-Biopharm AG, Darmstadt, Germany). It is based on the detection of soluble antigens of *Giardia lamblia* cysts and trophozoites in stool specimen. Here, *Giardia*-specific antibody is coated on the surface of the well of microtiter plate. Then stool sample is added followed by addition of conjugate. If *Giardia lamblia* is present in the specimen, a sandwich complex forms which is made up of the immobilized antibodies, the *Giardia lamblia* antigens and the conjugated antibodies. Unattached enzyme-labeled antibodies are removed during the washing phase. In a positive test, upon addition of the substrate, its color changes from colorless to blue. Adding the stop
Dyab et al - A comparison of different methods used for Diagnosis of Giardia ...... reagent stabilizes the color yellow that quantitatively correlates with the amount of the antigens.

**Results**

**Diagnosis of G. lamblia in Stool Samples:**
Sixty stool samples were positive for *G. lamblia* out of the 200 examined. This meant that the overall prevalence of *G. lamblia* was 30%. Macroscopic stool examination showed that most of the positive samples were bulky, offensive, greasy and yellowish in color.

**Comparative evaluation of four diagnostic methods for detection of G. lamblia in human stool samples:**
Four methods were used to detect *Giardia* in the present study: direct microscopic examination, concentration technique, detection of *G. lamblia* antigen by using rapid solid phase qualitative immunochromatography and quantitative EIA RIDASCREEN Giardia test. Comparing the four methods and techniques (Table 1), amongst the positive cases of *Giardia lamblia*, maximum were detected by RIDASCREEN Giardia test (30%) followed by immunochromatography test (28.5%), microscopic slide prepared after formalin-ether concentration technique (25%) and direct wet mount examination (20%). RIDASCREEN Giardia test gave the best results and highest number of positive samples (n = 60), followed by the copro-antigen detection technique using immunochromatography (n = 57; Figure 1), microscopic slide prepared after formalin-ether concentration technique (n = 50) and lastly the direct examination (n = 40).

The differences in positivity rates were found to be statistically significant (P<0.05). Statistically, the sensitivity and specificity of the methods were significant. Finally, RIDASCREEN Giardia test is a rapid and effective method with high sensitivity and specificity and detects Giardia antigens in stool specimens even when the count of parasite is low. This reduces the chances of missing even the asymptomatic cases.

Data were collected, tabulated and statistically analyzed using SPSS 20.0 software.

Examples of *Giardia* cyst and trophozoite detected in stool samples are shown in Figure 2.

Table 1: Comparison between the three used diagnostic methods for detection of *Giardia* infection. Data shown are frequencies; n (%). Total n = 207.

<table>
<thead>
<tr>
<th>Method</th>
<th>Positive Detection n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct microscopy</td>
<td>40 (20%)</td>
</tr>
<tr>
<td>Concentration technique</td>
<td>50 (25%)</td>
</tr>
<tr>
<td>Immunochromatography</td>
<td>57 (28.5%)</td>
</tr>
<tr>
<td>ELISA by RIDASCREEN Giardia test</td>
<td>60 (30%)</td>
</tr>
</tbody>
</table>

Figure 1: Immuno-positive *Giardia* antigen immunochromatographic detection test (A) compared to negative one (B). The blue line is a control and the red line is a positive diagnostic result.
Figure 2: Example cysts and trophozoites of *G. lamblia* detected in stool samples. A & B: Cysts and trophozoites detected by saline wet mount (x100). C & D: Cyst and trophozoite detected by iodine (x100). E & F: Cyst and trophozoite detected by Lacto-Phenol Cotton Blue (x40).

**Discussion**

*Giardia lamblia* is one of the most common intestinal protozoa in the world<sup>15</sup>. This study was designed to evaluate the prevalence of human giardiasis in children attending the outpatient clinics of Children Hospital of faculty of Medicine, Assiut University, and comparing efficacy of diagnostic methods for detection of *G. lamblia* in children stool samples. 200 stool samples were collected during the period from January to August 2015 from children with complaints of diarrhea and other gastrointestinal symptoms. All samples were examined by Direct smear (Saline wet mounts, iodine wet mounts and LPCB wet mounts), concentration technique (formol-ether concentration technique), qualitative immunochromatographic assay and quantitative EIA RIDASCREEN Giardia test.

The present study showed that the infection rate of giardiasis in the examined children was 30% which was higher than a previously reported much lower prevalence (9.3%)<sup>16</sup>. Also it did not agree with a 11.4% prevalence reported in Libya<sup>17</sup>. However, the rate of *Giardia lamblia* in the present study (30%) was lower than the reported 62.2%, 44.59% and 38% prevalences recorded in Egypt, Kirkuk and eastern Ethiopia (Dire Dawa), respectively<sup>18-20</sup>. High prevalence of giardiasis reflects lower educational level to health hygiene among children, poor experience in toilet use, overcrowded families, water contamination with Giardia parasite, and more frequent asymptomatic carriers. The
fluctuation in the prevalence between studies could be due to difference in investigative approaches, season of sample collection, local availability of clean water, and socioeconomic status for subjected persons at the time of study\(^{(21)}\). Although the cheap laborious conventional microscopy (with or without concentration techniques) is still being recommended to diagnose infections caused by *G. lamblia*, its sensitivity is found to be low (50-70\%) even after multiple examinations\(^{(22)}\). Moreover, it is time consuming and sensitivity can be lower in chronic giardiasis\(^{(23,24)}\). Antigen detection assays for *G. lamblia* had proven to be very useful with the advantages of reduced labor and time required in its diagnosis\(^{(25)}\).

In the present study, microscopic examination revealed that 40 children (20\%) were infected with *G. lamblia*. As using concentration method can increase the sensitivity of cyst detection by 10-12\%\(^{(26)}\), in the present study 50 children (25\%) were detected infected with *G. lamblia* using this method. However, higher number of cases was diagnosed using *Giardia* soluble copro-antigen Immunochromatography detection test. The superior efficacy of *Giardia* copro-antigen test was proven in several previous works\(^{(27,28)}\).

ELISA testing for diagnosis of Giardiasis increases detection of positive cases and detect the parasite at low-level of infestation or even when absence in the microscopic fecal examination\(^{(25)}\). RIDASCREEN Giardia test is a recent FDA approved EIA which detects *Giardia lamblia* soluble antigen in stool samples. In our study, out of 200 patients, 60 were diagnosed positive for *Giardia lamblia* by RIDASCREEN Giardia test which was far better than the direct wet mount microscopy which detected only 40 positive cases of giardiasis. The sensitivity and specificity of EIA test in comparison with direct wet mount microscopy was found to be 100\% and 91.5\%, respectively. Also, the giardiasis detection Immunochromatographic test is a potentially useful test because of its simplicity and specificity. It can be done by non-laboratory staff or by less-experienced personnel in poorly-equipped laboratory. The test is rapid and can be performed in 10 min time. However, sensitivity was low with some brands\(^{(26-30)}\).

**Conclusion**

RIDASCREEN Giardia ELISA test is a rapid and effective method with high sensitivity and specificity and detects *Giardia* soluble antigens in stool specimens even when the count of parasite is low. This reduces the chances of missing even the asymptomatic cases. Therefore, the test can be incorporated into routine diagnosis and screening. The Immunochromatographic test is a potentially useful specific and rapid test and can be done by non-laboratory staff though with lower sensitivity.

**Limitations of the Study**

- Fund limitation prevented more sample testing.
- Fund limitation also prevented us from conducting molecular comparative studies using specific PCR test.

**Conflict of Interest:** The authors declared no conflict of interests.

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