Evaluation of immunochromatographic assay in diagnosis of *Giardia lamblia* infection in comparison to Microscopy

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Abstract

Background: *Giardia* is recognized internationally as a most important etiology of persistent and chronic diarrhea especially in children with significant morbidity and mortality rates . Hence, rapid diagnosis is essential

Objectives: To assess the diagnostic performance of Rida®Quick *Giardia* dipstick versus microscopy

Patients and Methods: A hundred fecal samples were collected from one hundred patients complaining of chronic diarrhea. All samples were investigated by Rida®Quick *Giardia* dipstick for coproa-antigen detection, and microscopically using concentration techniques

Results: The sensitivity, specificity, PPV, and NPV of Rida®Quick *Giardia* dipstick were 81.3%,96.3 %,95.1 % and84.9 respectively. There is no cross-reactivity with other intestinal parasites. Rida®Quick *Giardia* dipstick provide adequate sensitivity and specificity and give rapid results.

Conclusion: Rida®Quick *Giardia* is simple, rapid and has good sensitivity and specificity. Moreover, it does not require experienced personnel or special technical equipments. So, it can be used as an test in specific substitutional situations where the microscopic diagnosis of *Giardia* is limited due to time constraints, lack of microscopy experts, unavailability of appropriate equipments or when examining large populations as in outbreaks and epidemiological surveys

Keywords: *Giardia lamblia*; Immunochromatographic assay; Microscopy

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Introduction

Giardiasis is one of the most widespread pathogenic intestinal protozoal infection worldwide (**Selim *et al*., 2015),** affecting nearly 2% of adults and 8% of children in developed countries. Also estimates show that nearly 70% of the inhabitance in developing countries **(Codrean *et al*., 2020).** In Egypt, prevalence of giardiasis is 48% this reality makes Egypt a hyperendemic region according to the World Health Organization (WHO) criteria **(Hawash et al., 2015).**

Giardiasis caused by *Giardia lamblia.* It floats through friction with infected people and infection occur by ingestion of contaminated food or drinking contaminated water. Children and malnourished patient are the most common groups being infected by giardiasis **(Chifunda and Kelly 2019).**

Symptoms of giardiasis differ from intestinal symptoms as diarrhea, abdominal discomfort, nausea and mild weight loss to extraintestinal symptoms like fever, lymphadenopathy, urticaria, maculopapular rash, polyarthritis, and pulmonary infiltrate **(Parida *et al*., 2019).**

Giardiasis can lead to complications such as weight loss and dehydration from diarrhea. the contagion can also cause lactose intolerance. Children under 5 years old who complaining from giardiasis are at risk for malnutrition, which can interfere with their physical and mental development **(Parida et al ., 2019).**

The diagnosis of giardiasis is often based on microscopical detection of the organisms in stool samples. Yet, the method is time consuming and depends on the proficiency of an experienced microscopist **(Chakarova *et al*.,2010)**. Diagnosis via microscopic examination of stool and may therefore lose up to 50% of *Giardia* infections. Because of the sporadic shedding of the parasites, microscopic examination of three consecutive stool specimens were required to reach the sensitivity of over 90%. **(Shahat *et al* .,2017)**

A double antibody sandwich ELISA technique, using a chromatography purified antisera against *G. lamblia* could detect 100% of those infected with G. lamblia **(Hassan *et al* .,2002)**

Giardiasis also can be diagnosed by performing entroscopy through flexible tube runs into small intestine and take tissue sample, but it is invasive method**.(Shahat *et al*.,2017)** The Enzyme_Linked Immunosorpent Assay (ELISA) also is used, and its work depends on monospecific antibodies for detection of coproantigen. The ELISA is sensitive and specific tool especially for confirming microscopy negative suspected cases, but it is very expensive. So, the development of sensitive, easy, cost effective and rapid diagnosticmethodsas immunochromatograghic test is of great importance. **. (Shahat *et al* .,2017)**

Out of 200 stool samples, 60 specimens (30%) it was 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%.**(Dyab *et al.,2016)***

Patients and methods

This descriptive analytical study was conducted from Jan.2020 toDec2020. All parasitological procedures were performed in the research laboratory of Medical Parasitology Department, Faculty of Medicine, South Valley University.

Fecal samples:Hundred fecal samples were obtained from 100 patients complaining of chronic diarrhea and attending the outpatient clinics of tropical medicine departments in South valley University Hospital, Egypt. Each sample was divided into two parts; the first part was examined freshly without preservatives by Rida®Quick *Giardia* dipstick , the second part was preserved in sodium acetate-acetic acid-formalin (SAF) for concentration techniques, and microscopic examination.

Microscopy The preserved samples were submitted to sheather' and formalin-ethyl acetate concentration techniques according to Garcia **(Garcia, 2016)**

Immunochromatographic assay: Fresh fecal samples were tested for *Giardia* copro-antigen using Rida®Quick *Giardia* dipstick (R-Biopharm, Germany). The test was carried out according to the manufacturer's instructions. In brief, the test procedure involved the addition of 100 µl of the diarrheic stool to 1 ml buffer in a test tube. The mixture was left for at least 3 min at room temperature until a clear supernatant was formed. Next, 200 µl (4 drops) of the clear supernatant of the stool suspension was added to the test window in the test , and the results were read after 5 min. A specimen was considered as positive when control (blue colored) and test (red colored) lines were visible (regardless of color intensity), as negative if only the control line showed blue band, and as invalid if no blue band was visible at the control line.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows version 25.0 and Medcalc version 15.8.0. Chi-square ($\chi 2$) test and Fisher's Exact Test were used for comparison regarding qualitative variables as appropriate. The results were considered significant when P value < 0.05, and a 95% CI was calculated. Sensitivity, Specificity, PPV, NPV and accuracy for the Rida®Quick *Giardia* were calculated using the microscope as the gold standard test

Ethical considerations: The study was authorized by the Scientific Ethics Committee of the Faculty of Medicine, South Valley University. Consents were obtained from enrolled patients or their guardians before data and sample collection with a brief explanation of the procedure and the purpose of the study. All infected patients were provided with appropriate treatment

Results

In the present study we reported that, the age of patients ranged from 2 to 60 years, 39% of them were from urban areas and 61% of them were from rural areas and 40% of patients presented with watery diarrhea , 60% of them presented with semisolid stool and 63% of patients presented with abdominal pain (**Table.1**).

Comparison between results of microscopy and Rida®Quick *Giardia* revealed that 39 samples were positive by both methods (true-positive), 9 samples were positive by microscopy but negative by Rida®Quick *Giardia* (false-negative) and 2 sambles were positive by Rida®Quick *Giardia* only (false-positive) . Relation is highly significant (P<0.000) (**Table.2**).

No samples were positive by Rida®Quick *Giardia* dipstick with intestinal parasites other than *Giardia* and this indicate that there is no cross-reactivity with other parasites' copro-antigens

Results showed that the sensitivity and specificity of Rida®Quick *Giardia* were (81.3%) and (96.3%) respectively (**Table .3**)

Table.1. Demographic and clinical data of the included patients

Variables	Frequency	Percent
Age	Mean ±SD 16.8±8.2	
Residence • **Urban** • **Rural**	 39 61	 39% 61%
Consistency of stool • **Watery** • **Semisolid**	 40 60	 40% 60%
Abdominal pain • **Present** • **Absence**	 63 37	 63% 37%

Table.2. Comparison between results of microscopy and *Rida®Quick Giardia*

Variables			**Microscopy**		***P* value**
			Positive	Negative	
Rapid test	**Positive**	Count	39	2	000*
		% within microscopy	81.3%	3.8%	
	Negative	Count	9	50	
		% within microscopy	18.8%	96.2%	
Total		Count	48	52	
		% within microscopy	100.0%	100.0%	

Table.3. Validity of rapid test (*Rida®Quick)* in diagnosing *Giardia*

Variables	Rapid test
Sensitivity	81.3%
Specificity	96.3%
Positive predictive value(ppv)	95.1%
Negative predictive value(npv)	84.9%
accuracy	89%

Discussion

Diarrhea is one of the most important causes of morbidity and mortality in developing countries, specially in malnourished children, patients with chronic diseases and immunocompromised **(Kotloff,2017).** *Giardia duodenalis* was considered as causative agent of diarrheal disease world- wide **(Efstratiou *et al*, 2017).** This parasite could be transmitted through contaminated water or foods, person to person, and by zo- onotic transmission **(Thompson and Smith 2011).**

The diagnosis of *Giardia* is usually recognized by microscopic detection of its trophozoites or cysts in stool specimens using different concentration techniques. Many epidemiological studies suggested that microscopy is time-consuming and requires an experienced personnel to identify the cysts **(Van *et al*., 2015).** Also, the number of cysts passed by patients varies from day to day and from week to week due to sporadic cyst excretion **(Sadaka *et al*., 2015).** So that, it must be performed on three stool samples to increase the sensitivity and this may limit the early diagnosis and treatment **(Van et al., 2015)**. Therefore, a number of copro-antigen assays have been developed for *Giardia* diagnosis including the immunochromatographic tests (ICT) **(Sadaka 2015)**

This study aimed to compare the commercially available rapid chromatographic Rida®Quick *Giardia* with the conventional diagnostic method using microscope. Ahundred stool samples were collected from patients complaining of chronic diarrhea, attending in Qena university hospital and Qena general hospital. Age ranged from 2 to 60 years (Age Mean ± SD =16.8±8.2),).

All samples were investigated by Rida®Quick *Giardia* dipstick for copro-antigen detection, and microscopically using concentration techniques ..

In our study, microscopic examination of smears was considered the gold standard to which results of Rida®Quick *Giardia* dipstick were compared

While it has been shown that microscopy, not 100% accurate in diagnosing *Giardia*, it is generally accepted as the gold standard against which new tests compared, and it has the advantage of being cheap compared to antigen detection tests. It also

could detect parasites other than *Giardia* when present in the stool samples **(Adeyemo *et al*., 2018)** In the present study infection was higher in patients living in rural areas, but without significant differences,and this agree with (**Forsel *et al.,* (2016**) reported that children in rural are- as were more susceptible to *G. lamblia* infection than those living in urban **.**(**Hawash *et al.,* (2015)** On the other hand, (**Ahmed *et al*., (2013)** in Gharbia Governorate found high prevalence of *G. lamblia* in urban than rural communities. The high percent of intestinal protozoan infections in rural areas may be due to poverty, poor living and hygienic conditions, drinking of underground water, which is contaminated with sewage, com- pared to urban areas, also the extensive use of human and animal excreta as fertilizer in agriculture, the household wastewater is thrown in irrigation channels in addition to the close contact with animals **(Pham-Duc *et al*., 2011**).

. In the present study, diarrhea and this agreed with (**Hawash *et al.,*2015**) who reported stated that acute and transient diarrhea in 71% of intestinal protozoan infection.

Of the 48 *Giardia* -positive samples that detected by wet smear and concentration methods , only (39) samples were positive by Rida®Quick *Giardia* dipstick so that they were considered as true-positive. Whereas the other nine samples were negative by Rida®Quick *Giardia* and they were considered as false-negative

. Based on the true-positive samples, the sensitivity of Rida®Quick *giardia* in our study was (82%). This agrees with **(Autier *et al*., 2018**) who reported a sensitivity of (88.2%

In contrast **Goñi *et al*., (2012**) reported lower sensitivity of (72.7%)

On the other hand, higher sensitivity reported by other studies such as **Regnath *et al*., (2006**) study that reported (100%) sensitivity

. The false-negative samples may be attributed to the presence of low parasite numbers which in turn leads to a drop in the antigen levels below the detection limit of the rapid methods **(Sadaka *et al*., 2015).**

On the other hand, there are (50) true-negative samples that were negative by both methods. Whereas 2 were considered as false-positive as it was positive by Rida®Quick *giardia* but negative by microscope

. The specificity of Rida®Quick *Giardia* in the present study was (96.3)%. This agrees with Goñi *et al*., (2012) who reported a specificity of (95.7%)

On the other hand, many studies reported 100% specificity as **Regnath *et al*., (2006), Weitzel *et al*., (2006)**

The variability in the sensitivity and specificity of Rida®Quick *Giardia* from one study to another may be attributed to the type of the "gold standard" test to which the results compared Rida®Quick *Giardia* didn't reveal positivity for any stool samples containing intestinal parasites other than *Giardia* . This means there is no cross-reactivity with other parasites' copro-antigens. This agrees with **Regnath *et al*., (2006) and Weitzel *et al*., (2006).**

Regarding the simplicity of technique, Rida®Quick *Giardia* was simple to be performed and required minimal technician training. In contrast, microscopic examination needed multiple steps for concentration . Also, recognition of the trophozoites and cysts required training and practice

Considering the time consuming, The time required for the performance of Rida®Quick *Giardia* and microscopic examination varied considerably.Time needed for microscopy took average 2 minutes to prepare the sample, 20 minutes of centrifugation, 3 minutes for fecal smear preparation). Rida®Quick *Giardia* required much less time (10 minutes or less).

With regard to cost, the cost of Rida®Quick *Giardia* is high and it is one-use. Whereas the microscopy requires the availability of microscope, centrifuge, materials for concentration techniques that are not always easy to purchase, especially in laboratories with limited resources.

Limitations of this study were the small sample size because of cost, and the tests employed for the diagnosis of *Giardia* did not include more accurate methods such as PCR.

Moreover, failure to characterize the *Giardia* species using molecular techniques in those false-negative samples might be a missed opportunity to better elucidate the findings in a way to offer input towards continuous improvement of the rapid tests

Conclusion

The present data revealed that microscopy is still the preferred method for diagnosing *Giardia*; being more sensitive, of low cost and it allows detection of other intestinal parasites in the sample if present.

We found that Rida®Quick *Giardia* is simple, rapid and has good sensitivity and specificity. Moreover, it does not require expert personnel or special technical equipment.

Conflict of Interests

The authors report no conflict of interest.

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