IMMUNOCHROMATOGRAPHY VERSUS MICROSCOPY FOR DIAGNOSIS OF *ENTAMOEBIA HISTOLYTICA/DISPAR* INFECTION IN SOHAG UNIVERSITY HOSPITALS, EGYPT

By

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Abstract

Amoebiasis caused by *E. histolytica* protozoon, is a major public health worldwide. Delay in diagnosis and treatment may lead to fatality. This study evaluated the efficacy of immunochromatographic test (ICT) using RIDA®QUICK *Entamoeba* test compared with traditional microscopic examination for *E. histolytica /dispar* infection. A cross-sectional study was conducted on 100 patients suffering from dysentery or diarrhea with an age range of 3-62 years, attended Endemic Diseases Hospital, Sohag University Hospitals. Stool samples were examined by direct wet smears, formalin ethyl acetate concentration methods, stained with Wiegert's iron hematoxylin and Modified Ziehl-Neelsen stains. Microscopic examination showed 43/100 (43%) patients having *E. histolytica/dispar*. Copro-antigen detection in stool sample was done using Rida®Quick *Entamoeba* test. The sensitivity, specificity, PPV & NPV of Rida®Quick *Enta-moeba* tests were 97.67%, 96.49%, 95.45% & 98.21% respectively with an accuracy of 97.7%, and without cross-reactivity with some endemic intestinal parasites. The allover infection rate was significantly associated with residence (P < 0.036) and family size (P < 0.03), but neither associated with sex (P < 0.471) nor age (P < 0.344). Rida® Quick *Entamoeba* test proved to be simple, rapid, convenient, sensitive and specific assay to diagnose *E. histolytica /dispar* infection for epidemiology.

Keywords: Rida®Quick *Entamoeba* test, ICT, Microscopy, *Entamoebia histolytica/dispar.*

Introduction

Entamoeba histolytica is an anaerobic parasitic amoebozoan, of genus *Entamoeba* that infects about 35-50million people worldwide, especially in tropical areas with poor sanitary conditions (Rawat *et al*, 2020), and annually killed more than 55,000 patients (CDC, 2017). Stauffer *et al.* (2006) in Egypt reported endemic *E. histolytica* with high rates of asymptomatic infection detected in stool (>21%). Transmission of *E. histolytica* occurs by fecal excretion of cysts followed by oral ingestion of contaminated food or water, but fecal-oral transmission may occur within households and long-term care institutions (Thompson and Smith, 2011).

In ameobiasis, 90% of infected patients were asymptomatic; others have symptoms ranged from colitis to dysentery and extra-intestinal amebiasis (Fotedar *et al*, 2007). The commonest clinical extra-intestinal infection is amoebic liver abscess and less co-mmon or rare pleuropulmonary involvement and cardiac infection (Stanley, 2003).

Laboratory diagnosis of *E. histolytica/dispar* is usually by microscopy stained smears, may be on two or three successive days or more. So, it may be time-consuming and requires an experienced technician (Carrero *et al*, 2020). Antigen detection assays such as rapid immuno-chromatographic tests (ICT) for *E. histolytica* were developed for diagnosing infection, without microscopy skills and increase laboratory efficiency by reducing time (Saidin *et al*, 2018).

This study aimed to evaluate ICT by using the Rida®Quick *Entamoeba* test compared with traditional microscopy for copro-antigen detection of *E. histolytica/dispar*.

Materials and Methods

Study area: This study was carried out in Sohag Governorate, Upper Egypt. Sohag is located in the southern part of the country toward 467km to the south of Cairo. It co-

vers an extent of the Nile Valley with a total area of 1547 km^2, with estimated 5,510,510 people.

Study design: A cross-sectional study was conducted on 100patients suffered from dysentery or diarrhea attending the Endemic Diseases Hospital, Sohag University Hospitals from March 2021 to January 2022. They had to fulfill the criteria; not on amebic drug 2 weeks before sample collection. They were subjected to a detailed medical questionnaire.

Ethics approval: The study was approved by the Medical Research Committee of Faculty of Medicine, Sohag University, with the IRB registration No (Soh-Med-21-02-16). This study was registered on 18/2/2021, with registry No. (NCT04759937). All meth ods were performed in accordance with the relevant guidelines and regulations, which when with Helsinki (2000) Written informed consent was obtained from each patient after explaining study the procedure and purpose.

Laboratory examinations: Fecal samples were collected in labeled clean, covered plastic containers. Each sample was divided into two parts; one part was examined macros copically for consistency, color, odor, blood and mucus, adult worms or segments and then microscopically by: a- direct wet mount saline smear and Lugol's iodine smear (Garcia and Bruckner, 1997), b- formalin ethyl acetate sedimentation and c- stained smear with Wiegert's iron hematoxylin and modified Ziehl-Neelsen stains (Garcia and Bruckner, 1997). The slides were examined using low and high powers (Cheesbrough, 2009), Second part was examined by rapid ICT Rida®Quick *Entamoeba* test (R-Biopharm AG, Darmstadt, Germany, LOT NO AL57.43) as the manufacturer's instructions given

Immunochromatography assay: Fresh fecal samples without preservatives were used to do the test. Test procedure involved adding 100μl (50mg) of the diarrheic stool to 1ml buffer in a test tube, and left for at least 3 min at room temperature to have a clear supernatant. Then, 200μl (4drops) of the supe-

rnatant was placed into the round opening of the cassette and result was read after 5 min. Sample was considered positive when control (blue-colored) and test (red-colored) lines were visible (regardless of color intensity), or negative if only control line showed a blue band, and as invalid if no blue band was visible at the control line, but a weak pink line as trace result.

Statistical analysis: Data were analyzed by IBM SPSS Statistics for Windows version 25.0 and Medcalc version 15.8.0. Quantitative data were expressed as mean ± SD. Qualitative data were expressed as number and percentage. Chi-square test and Fisher's Exact Test were used for comparison regarding qualitative variables as appropriate. Sensitivity, Specificity, PPV, NPV, and accuracy were calculated to evaluate the kits with microscopy as the gold standard. For measuring the agreement between microscopy and Rida® Quick *Entamoeba* test. Cohen's kappa test was done with significance levels of: ≤ 0= poor, 0.01-0.20 = slight, 0.21-0.40= fair, 0.41-0.60= moderate, 0.61-0.80= substantial and 0.81-1= almost perfect. *P*-value was considered significant if it was < 0.05.

Results

The patients 46% were males and 54% females, ages ranged from 3 to 62(20.44±1.61) years, 73% live in rural areas and 27% in urban ones, & 65% with family size ≥ 5 members but, 35% < 5 members. *E. histolytica/dispar* by microscopy was in 43/100(43%).

Of 43 patients with *E. histolytica/dispar* 30 patients were with single infection, seven patients co-infected with *Giardia lamblia*, five patients with *Blastocystis hominis* and one patient with *E coli*. Others were *Cryptosporidium parvum* (25%), *Giardia lamblia* (16%), *Cyclospora* spp. (5%), *Blastocystis hominis* (5%), *Entamoeba coli* (3%), *Iodamoeba buetschilii* (2%), *Hymenolepis nana* (1%) and *Enterobius vermicularis* (1%).

The infection was significant with residence (P < 0.036) & family size (P < 0.03), but neither significant sex (P < 0.471), nor age (P < 0.344).

Details were given in tables (1, 2 & 3) and   figures (1 & 2).

Table 1: Sociodemography of *E. histolytica/dispar* among 100 outpatients

Variations	Positive (n= 43)	Negative (n= 57)	*P* value
Age: <20 years	25 (58.14%)	36 (63.16%)	
20-40 years	15 (34.88%)	12 (21.05%)	
>40-60 years	3(6.98%)	7 (12.28%)	0.344
>60 years	0 (0%)	2 (3.51%)	
Male	18 (41.86%)	28 (49.12%)	0.471
Female	25 (58.14%)	29 (50.88%)	
Residency: Rural	36 (83.72%)	37 (64.91%)	0.036*
Urban	7 (16.28%)	20 (35.09%)	
Family size: < 5 members	8 (18.6%)	27 (47.37%)	0.03*
≥ 5 members	35 (81.4%)	30 (52.63%)	

* Significant= P< 0.05.

Table 2: Efficacy of RIDA®QUICK *Entamoeba* test in comparison to microscopic examination

Rida® Quick *Entamoeba* cassettes	Microscopy			Sensitivity%	Specificity%	PPV %	NPV %	Accuracy %
	Positive	Negative	Total					
Positive	42%	2%	44%					
Negative	1%	55%	56%	97.67	96.49	95.45	98.21	97.67
Total	43	57	100					

P value = .0000*, Kappa = 0.939**

Table 3: Microscopic examination versus RIDA® QUICK *Entamoeba*

Microscopic results	Rida®Quick positive	Rida®Quick negative	Positive %
E. histolytica/dispar (n = 43)	42	1	97. 7%
Cryptosporidium parvun (n = 25)	0	25	0.0%
Giardia lamblia (n = 16)	0	16	0.0%
Cyclospora (n = 5)	0	5	0.0%
Blastocystis hominis (n = 5)	0	5	0.0%
Entamoeba coli (n = 3)	0	3	0.0%
Iodamoeba buetscilii (n = 2)	0	2	0.0%
Hymenolepis nana (n = 1)	0	1	0.0%
Enterobius vermicularis (n = 1)	0	1	0.0%

Discussion

In the present study, Rida®Quick *Entamoeba* results versus microscopy showed that 42 samples were positive by both (true-positive), one sample was positive by microscopy, but negative by the test (false-negative) and two samples were positive only by test (false-positive), with significant (P< 0.0001) Kappa value was 0.939, with perfect agreement between both microscopy and the test. In the present study, sensitivity, specificity, PPV & NPV of Rida® Quick *Entamoeba* cassettes were 97.67%, 96.49%, 95.45% & 98.21% respectively.

The Rida® Quick *Entamoeba* cassettes didn't show any cross-reactive with any intestinal parasites other than *E. histolytica/dispar*. Besides, several authors reported that microscopy was labor-intensive, time-exhaustion, needs technician's experience, and with low sensitivity (Vanathy *et al*, 2017). Also, the examination of three stool samples over not

more than 10 days is recommended to increase microscopy sensitivity, as such parasites may be passed intermittently (Varghese *et al*, 2021). So, Immunochromatographic tests were accepted to overcome the disadvantages and allow the detection of *Entamoeba* copro-antigen (Saad *et al*, 2015).

In the present study, the microscopic examination showed that prevalence of *E. histolytica/dispar* was 43% among the patients. This more or less close agreed with that reported in Egypt; 40.77% among 130 patients with chronic abdominal pain in Sohag City (Omran and Mohammed, 2015) and 44.4% among 230 primary school children in Zagazig City (Hussein *et al*, 2021).

Dyab *et al*. (2016) in Aswan Governorate among children found the commonest protozoa infection was *E. histolytica* 8.3% followed *by G. lamblia* 3.7% and *C. parvum* 1.7% and that the parasitosis was more prevalent in boys (53.8%)

than girls (46.2%). El-Nadi *et al.* (2017) among school children in Sohag Governorate found that protozoa were more common than helminthes (53.5% vs. 4%) with *Cryptosporidium* spp., *G. duodenalis* and *E. histolytica/dispar* prevalences were 34%, 14%, & 13% respectively. They added that a significant difference was detected between diarrhea and polyparasitzed children. El-Nadi *et al.* (2019) in Sohag by using the mini-FLOTAC for the diagnosis of intestinal helminths and protozoa, they suggested that it was a valid, sensitive and relatively low-cost alternative technique that could be used in resource-limited settings, particularly for helminthic diagnosis.

The present study showed the highest prevalence was among the age group less than 20 years (58.14%), followed by patients between 20-40 years (34.9%) and patients >40-60 years (6.98%), but without significance between age and infection (P= 0.344). This agreed with El-Nadi *et al.* (2017) in Sohag and Salem *et al* (2017) in Libya who didn't find significant relation between infection and children age groups (P < 0.425, P < 0.081 respectively). But, this disagreed with Hegazi *et al* (2013) in Saudi Arabia who reported that the infection prevalence among infants less than one year was significant (P < 0.02) due to inadequate breastfeeding and bottle-feeding practices among cases.

The present study showed *E. histolytica/dispar* infection among females (58.14%) was higher than males (41.86%), however, without significant association between the sexes and the infection (P < 0.471). This agreed with El-Nadi *et al* (2017) who didn't find significant differences between sexes (P < 0.446). However, this disagreed with Dyab et al. (2016) and Salem *et al* (2017**)** who found boys were significantly more infected than girls.

In the present study, patients in rural areas (83.72%) were at higher risk for *E. histolytica/dispar* infection than those living in urban ones (16.28%) with significant difference (P < 0.036). This agreed with Dyab *et al.* (2016) in Egypt and Khan *et al* (2019) in Pa-

kistan who reported that prevalence of parasitosis was significantly high in rural areas than urban ones (P < 0.05). But, Atia *et al.* (2016) in Zagazig City found that the infection rate was higher in patients in rural areas, but without significance (P >0.05). Generally speaking, soil-transmitted parasites in the rural areas may be due to poverty, poor ecological and hygienic conditions compared to urban ones (Duc *et al*, 2011).

In the present study, the patients with large family sizes (81.4%) showed a significantly higher prevalence of the infection than those with small size (18.6%), with significant difference (P = 0.03). Intestinal parasites were prevalent in children from overcrowded families and without knowledge of IPIs. Educative programmes on IPIs, improving hygiene, and application of supportive programmes to elevate socioeconomic conditions may help reduce the burden of intestinal parasite carriage in children.

In the present study, the sensitivity, specificity, PPV, & NPV of the Rida®Quick Entamoeba test was 97.67%, 96.49%, 95.45% and 98.21% respectively with no cross-reactivity with other intestinal parasites. This agreed with Saad *et al* (2015) who revealed sensitivity (100%) and specificity (97.4%) in addition to Atia *et al* (2016) who reported sensitivity (100%) and specificity (100%). As these studies compared the ICT with microscopy in detection of the parasites in stool samples.

However, these results disagreed with a study by Goñi *et al* (2012) who reported lower results in detection of *E. histolytica* where sensitivity was 62.5% and specificity was 96.1% which might be due to the fact that they used PCR as standard reference. Also, Abu Sheishaa (2021) found lower sensitivity (80.0%) and specificity (88%) as they used ELIZA as standard reference.

The false-negative samples may be attributed to the presence of low parasite numbers, which leads to a drop in the antigen levels below the detection limit of the rapid methods (Garcia *et al*, 2003; Weitzel *et al*, 2006).

On the other hand, the false-positive samples may be due to intermittent parasite excretion in the stool or due to persistent antigen in recently cured patients (Shimelis and Tadesse, 2014). However, the limitations of the ICT test were its high cost and inability to differentiate between pathogenic *E. histolytica* and non-pathogenic *E. dispar*. Moreover, quantitative ICT models are required to measure the intensity of infection and monitor therapeutic success. Nevertheless, Calle-Pacheco *et al.* (2022) reported that the mole cular diagnosis of amoebiasis is a must as an innovative tool in fight against this parasite.

Conclusion

Rida®Quick *Entamoeba* test is simple, rapid, with high sensitivity and specificity. It can be used in specific cases where microscopic diagnosis of *E. histolytica/dispar* is limited and epidemiological surveys. However, microscopy failed to detect only one case

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Explanation of figures

Fig 1: Results of Immunochromatographic Rida®Quick *Entamoeba* tests: A. Positive, B. negative, and C. trace.
Fig. 2: Immunochromatographic Rida®Quick *Entamoeba* cassettes (R-Biopharm, Germany, LOT NO. AL57.43)