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COMPARISON OF IMMUNOCHROMATOGRAPHIC TEST AND MICROSCOPY IN THE DETECTION OF SOME ENTERIC PROTOZOA IN STOOL SAMPLES

By

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Abstract

Infection with pathogenic intestinal protozoa as *Giardia lamblia, Entamoeba histolytica* and *Cryptosporidium parvum* cause considerable gastrointestinal morbidity, malnutrition and mortality worldwide, especially among young children in developing countries. The present study was carried out on 71 cases (44 males & 27 females) chosen from Pediatric and Internal Medicine Inpatient and Outpatient Clinics of Zagazig University Hospitals, complaining of different gastrointestinal troubles with an age range of 6-60 years. Also, 20 apparently healthy individuals (11 males & 9 females) cross matched were considered as a control negative group. All stool samples were examined by direct wet smears, concentration techniques, staining of the smears using trichrome stain and Modified Ziehl-Neelsen method. Copro-antigen detection in faecal sample was by using quick immunochromatographic test. A total of 71 cases suffering from different GIT manifestations showed *G. lamblia* (30.7%), *Cryptosporidium parvum* (19.8%), *Entamoebahistolytica/E. dispar* (11%) and mixed infection of three protozoa (6.6%). However, by copro-antigen *G. lamblia* was positive in (31.8%) of *C. parvum* in (20.9%); *E. histolytica/E. dispar* in (11%) of cases. Immunochromatography/copro-antigen test recorded sensitivity and specificity of (100%) and (96.6%) respectively in *G. lamblia* detection. For *C. parvum*, sensitivity was (100%) and specificity was (97.1%) while for *E. histolytica/E. dispar*, sensitivity and specificity were (100%) for both. Immunochromatographic assay proved to be simple, easy and useful in confirming absence or the presence of intestinal protozoan infection in clinically suspected cases with negative stool examination.

Keywords: Egypt, Zagazig, Patients, Immunochromatographic test, Microscopy, Intestinal protozoa

Introduction

Diarrheal diseases were ranked the second cause of morbidity and mortality in children in the developing countries (WHO, 2005), as a major cause of morbidity and mortality worldwide particularly where poor sanitary and hygienic conditions exist (Walker *et al,* 2012). Diarrhea is defined by having three or more loose or liquid stool per day, it can be caused by different bacteria, viruses and parasites and can spread through contaminated food, drinking water or from person to person as a result of poor hygiene (WHO, 2014). The most common parasitic causes of acute diarrhea are the intestinal protozoa of which *Entamoeba histolytica*; *Giardia lamblia* and *Cryptosporidium parvum* are considered the most important (Kourenti *et al,* 2007).

G. lamblia is the commonest cause of waterborne diarrhea worldwide. Although, it causes as a self-limiting acute infection, but evidence suggested that acute giardiasis may lead to the development of chronic functional gastrointestinal disorders, such as post-infectious irritable bowel syndrome (IBS) and functional dyspepsia, by unknown mechanisms (Spiller and Garsed, 2009; Cotton *et al,* 2011). *E. histolytica* have a worldwide distribution, particularly in tropical and subtropical areas, and is one of the leading parasitic burdens in developing countries, contributing to 50 million cases and an estimated 100,000 deaths annually (Aguilar-Diaz *et al,* 2011; Morf and Singh, 2012). *C. parvum* is a zoonotic protozoan parasite, which oocysts are resistant to chlorinated water and can survive in aquatic environ-

ments for a long time (Chauret *et al,* 1998). The association of *Cryptosporidium* with waterborne-related outbreaks of diarrhea gave parasite a risky importance (Shaapan and Khalil, 2014).

Although microscopic examination of stools for detection of cysts, oocysts and trophozoites remains the diagnostic method of choice, as it is simple and of low cost, it requires technical expertise, time consuming and does not allow determination of the parasite species or genotype (Ryan and Cacciò, 2013). Antigen detection assays, such as enzyme immunoassays (EIAs) and immunochromatography (IC), for detection of *C. parvum, G. lamblia* and *E. histolytica/dispar* were developed (Garcia *et al,* 2003). The triple immunochromatographic tests proved to be a simple, fast and additive method for the simultaneous diagnosis of these parasites in stool samples. Also, it requires little exercise and can be used in individual base for timely screening (Weitzel *et al,* 2006).

The present study was designed to evaluate the sensitivity and specificity of the triple immunochromatographic test in relation to direct microscopic examination for detection of most common enteric protozoa (*G. lamblia, C. parvum &E. histolytica*/*E. dispar*) in stool samples of GIT patients.

Subjects, Materials and Methods

The present work was carried out on 71 cases (44 males and 27 females) attending Pediatric and Internal Medicine Inpatient and Outpatient Clinics of Zagazig University Hospital, and complaining of different gastrointestinal troubles with an age range of 6-60 years. Also 20 healthy cross-matched individuals (11 males & 9 females) were utilized as control negative group. For all (patients and controls), stool samples were collected on three consecutive days. Each sample was divided into 2 parts one kept fresh for stool examination and the other was kept at (-20) for antigen testing by the quick immunochromatographic test.

Stool examination: Each sample was examined macroscopically (consistency, colour, odour and the presence of blood or mucous) and microscopically by direct smear examination both saline and iodine wet mounts (Fleck and Moody, 1988), Zinc sulphate centrifugal floatation (Beaver GIT, 1984), Formol-ether sedimentation (Cheesbrough, 2009), the slides were examined using a low-power objective (10×) and high-power objective (40×) respectively. Some smears prepared from formol ether techniques were fixed using Schaudinn's fixative to be stained later by modified Ziehl-Neelsen method (Cheesbrough, 2000), and trichrome staining technique (Cheesbrough, 1987).

Quick immunochromatographic test (RIDA®QUICK *Cryptosporidium*/ *Giardia*/ *Entamoeba* Combi): It is a single-step immunochromatographic test, where specific antibodies against each parasite attach themselves to green (*Entamoeba* specific), red (*Giardia* specific) or blue (*Cryptosporidium* specific) latex particles. Band appears, depending on the antigens present in the sample. According to the manufactures guide, Stool samples and all reagents were brought to room temperature before use, each sample (100µl from liquid stool or 50mg from solid stool samples) was well mixed with one ml from the extraction buffer using the vortex mixer, and then they were left for at least three minutes to allow the homogenized suspension to precipitate. From the clearly formed supernatant 200µl-500µl were transferred into other clean tubes. A test strip was immersed in each tube. The test result can be read off after 10 minutes. A maximum of four bands should appear in the following order from the sample-absorption site: blue, red, green and crimson (control). If the control band is missing, the test is invalid and cannot be evaluated.

Statistical analysis: Data were recorded, calculated, tabulated and statistically analyzed using statistical computer program SPSS version 16 under windows 7.

alone. GIV: Patients with mixed infection of the three protozoan parasites. GV: patients negative for these protozoan parasites but infected with other parasites. GVI: Healthy group as control who were negative for all parasites.

Results

According to microscopic examination cases were divided into six groups as follow: GI: patients infected with *G. lamblia* alone. GII: patients infected with *C. parvum alone.* GIII: patients infected with *Entamoeba*

Table 1: Incidence of three protozoan parasites according to age among corresponding group

Age	GI (n=28)		GII (n=18)		GIII (n=10)		GIV (n=6)		χ^2	P
	N	%	N	%	N	%	N	%		
6-15	17	60.7	9	50	5	50	4	66.7	3.20	0.78 NS
16-30	7	25	6	33.3	2	20	2	33.3		
31-60	4	14.3	3	16.7	3	30	0	0		
$\chi^{2\#}$	9.93		3		1.4		0.67			
P	0.007*		0.22 NS		0.50 NS		0.42 NS			

Table 2: Incidence of three protozoan parasites according to sex among corresponding group.

Sex	GI (n=28)		GII (n=18)		GIII (n=10)		GIV (n=6)		χ^2	P
	N	%	N	%	N	%	N	%		
Male	18	64.3	10	55.6	6	60	3	50	0.61	0.90 NS
Female	10	35.7	8	44.4	4	40	3	50		

Table 3: Incidence of three protozoan parasites according to residence among corresponding group.

Residence	GI (n=28)		GII (n=18)		GIII (n=10)		GIV (n=6)		χ^2	P
	N	%	N	%	N	%	N	%		
Rural	18	64.3	12	66.7	6	60	3	50	0.60	0.89 NS
Urban	10	35.7	6	33.3	4	40	3	50		

Table 4: Comparison between microscopic examination and immunochromatographic test (ICT) among groups:

Variable	Microscopy (n=91)		ICT (n=91)		Kappa	P
	No	%	No	%		
Giardia lamblia	28	30.7	29	31.8		
Cryptosporidium parvum	18	19.8	19	20.9		
Entamoeba histolytica/E. dispar	10	11	10	11		
Mixed	6	6.6	7	7.7		
G. lamblia + C. parvum	2	2.2	3	3.3		
E. histolytica/E. dispar + G. lamblia	1	1.1	1	1.1		
E. histolytica/E. dispar + C. parvum.	2	2.2	2	2.2		
E. histolytica/E. dispar +G. lamblia + C. parvum.	1	1.1	1	1.1		
Others	9	9.9	0	0	0.82	<0.001**
Entamoeba coli	3	3.3	-			
Blastocystis hominis	3	3.3	-			
Enterobius egg	2	2.2	-			
Srongyloides stercoralis larva	1	1.1	-			
Negative	20	22	26	28.6		

Table 5: Validity of ICT in diagnosis of *Giardia lamblia* in comparison to microscopy as Gold standard:

ICT:	Microscopy		Total
	+ve	–ve	
+ve	32	2	34
-ve	0	57	57
Total	32	59	91
Validity	Sensitivity:100% PPV: 94.1%	Specificity: 96.6% NPV: 100 %	
Accuracy	97.8%		

Table 6: Validity of ICT in diagnosis of *C. parvum* infection in comparison to microscope as Gold standard**:**

ICT:	Microscopy		Total
	+ve	–ve	
+ve	23	2	25
-ve	0	66	66
Total	23	68	91
Validity	Sensitivity:100% Specificity: 97.1% PPV: 92% NPV: 100 %		
Accuracy	97.8%		

Table 7: Validity of ICT in diagnosis of *E. histolytica/E. dispar* i in comparison to microscope as Gold standard:

ICT:	Microscopey		Total
	+ve	–ve	
+ve	14	0	14
-ve	0	77	77
Total	14	77	91
Validity	Sensitivity:100% Specificity: 100% PPV: 100% NPV: 100 %		
Accuracy	100%		

Discussion

Diarrheal diseases are one of the causes of morbidity and mortality in developing countries, especially in malnourished children, patients with chronic diseases and immunocompromised (Abdel-Hafeez *et al,* 2012). *Cryptosporidium*, *Giardia duodenalis* and *Entamoeba histolytica* were recognized as causative agents of diarrheal disease worldwide (Stark *et al,* 2009). These protozoa could be transmitted through contaminated water or foods, person to person, and by zoonotic transmission (Thompson and Smith, 2011).

In the present study, children were 6-15 years old recorded the highest infection percentage (60.7%) followed by 16-30years old (25%) and then 30-60 years (14.3%) with significant difference and males (64.3%) were more than females (35.7%), but without significant difference (p >0.05). These agreed with Helmy *et al.* (2009) who found that the highest percentage of infection in patients 10 to 20 years old (56.3%) among 41 Egyptian patients with *G. lamblia* aged between 0-65 years old. Bernawi *et al.* (2013) also found that the highest infection rate was in the same age group with males were commonly affected more than females without significant difference. This could be explained by this group are fully independent in toilet use and more involved in outdoor activities which might lead to *Giardia* transmission. The result agreed with worldwide reports suggesting that giardiasis is one of the major health problems among population of younger age groups (Anuar *et al,* 2012). In contrast, De Lucio *et al.* (2015) found that the highest infection rate of symptomatic giardiasis was in the age group 0-4 years, and that males were affected more than females. This could confirm that infants and young children are most susceptible to the infection and males are commonly affected most probably due to their wide range of movement in the society. On the other hand, Zaglool *et al.* (2011)in Saudi Arabia stated that the giardiasis incidence was equal in both sexes.

In the present study infection was higher in patients living in rural areas, but without significant difference (p >0.05). Almerie *et al.* (2008) reported that children in rural areas were more prone to *G. lamblia* infection than those living in urban areas, but without significant difference. Mbuh *et al.* (2011) in south-west Cameroon reported higher infection with *E. histolytica* in rural (34.0%) than urban areas (18.4%). Also, Mohammad *et al.* (2012) in Egypt reported that the prevalence of parasites was more common in rural areas. Mathew *et al.* (2014) in Nigeria found

that prevalence of *C. parvum* was higher among children in rural areas. On the other hand, Ahmed (2013) in Gharbia Governorate found high prevalence of *E. histolytica* and *G. lamblia* in urban than rural communities. The high percentage 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, compared 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 alone was found to be the commonest clinical symptom in patients with *G. lamblia* 50%, *C. parvum* 50% and mixed infection 100% while in *E. histolytica/E. dispar* group, diarrhea alone was found in 20% of cases with significant difference between them. This might be due to the fact that *G. lamblia* and *C. parvum* inhabit the small intestine, and thus expected to be present with diarrhea. This agreed with Hawash *et al.* (2015) who reported stated that acute and transient diarrhea in 71% of intestinal protozoan infection.

In the present study, stool samples were examined by wet smear, concentration techniques, staining with both trichrome and modified Ziehl-Neelsen and antigen detection using immunochromatography. Microscopic examination was taken as the gold standard and the sensitivity and specificity of the immunochromatography test was calculated in comparison with this fact. Microscopic examination of stool samples showed that the most frequent parasite detected was *G. lamblia* (30.7%) followed by *C. parvum* (19.8%) and then *E. histolytica/E. dispar* (11%). There were also mixed infections of the three protozoan parasites (6.6%). Parasites other than the parasites under study were found including *Entamoeba coli* (3.3%), *Blastocystis hominis* (3.3%), *Enterobius* (2.2%) and *Strongyloides stercoralis* (1.1%). The twenty samples (controls) were negative for all parasites.

Gaafer (2011) in Alexandria found that *G. lamblia* was the commonest followed by *C. parvum* and then *E. histolytica/E. dispar* among 100 patients attended the outpatient clinic of Alexandria University Hospitals. But, Goni *et al.* (2012) found that *E. histolytica/E. dispar* (25.63%) was the commonest followed by *G. lamblia* (19.38%) and then *C. parvum* (13.75%), which might be due to different in environmental conditions

In the present study none was positive by copro-antigen immunochromatographic test, that is to say no cross reactivity occurred with copro-anitgen with parasites other than three dealt with. Also, Gaafer (2011) reported that Triage Micro parasite Panel test did not diagnose any parasite other than *G. lamblia, C. parvum* and *E. histolytica/E. dispar* with no cross reactivity with other intestinal parasites. The triple ICT for *G. lamblia* showed that the sensitivity was100%, the specificity was 96.6%, the positive predictive value was (PPV) 94.1% and the negative predictive value (NPV) was 100%. Goni *et al.* (2012*)* reported that the sensitivity and specificity for the triple ICT were 96.8% and 99.5% respectively for *G. lamblia* detection. Also, Swierczewski *et al.* (2012) used triage parasite panel on 266 samples in Kenya and found that the sensitivity 100% and specificity 100% in detection of *G. lamblia*.

When using triple ICT for the detection of *C. parvum,* sensitivity was 100%, specificity was 97.1%, PPV was 92% and NPV was 100%. Regnath *et al.* (2006) obtained 100% sensitivity and specificity in *C. parvum* diagnosis with Rida®Quick *Crypto/Giardia* combi. Others, with the same test, in diagnosis *Cryptosporidium* and *Giardia* got 92% & 97% sensitivity (Gutiérrez-Cisneros *et al,* 2011). On the other hand, Goni *et al. (2012)* reported lower results in detection of *C. parvum* by the triage where the sensitivity was 72.7%. Swierczewski *et al.* (2012) found lower 73% sensitivity in *C. parvum* detec-

tion, which might be due to difference in the monoclonal antibodies used.

For *E. histolytica /E. dispar,* the triple ICT showed that the sensitivity was 100%, the specificity was 100%, PPV was 100% and NPV was 100%. This agreed with Swierczewski *et al.* (2012) in Kenya who mentioned that the sensitivity and the specificity were 100% when compare the triage ICT with microscopy in detection of the three parasites in stool samples. But, lower results reported by Goni *et al.* (2012), which might be due to the fact that they used PCR as standard reference.

A limitation to the Triple ICT as with microscopic examination is that both are unable to differentiate between the pathogenic *E. histolytica* and the nonpathogenic *E. dispar*, so this requires use confirmatory test to differentiate in between as ELISA & PCR.

Conclusion

The Triple ICT is sensitive and specific for the detection of *G. lamblia, C. parvum* and *E. histolytica/E. dispar.* The kit could be used as a screening tool in wide-scale prevalence studies or in suspected outbreak investigations because it is rapid and simple procedure and don't need training. The Triage Micro Parasite Panel could be used in conjunction with ordinary microscopic examination in medical laboratories or possibly as an alternative method.so we recommend their use in epidemiological studies in control programs.

Authors contribution: Atia MM, proposed the idea, Elsettawy MA and Fathy GM, shared in the design, reviewed practical work, wrote and reviewed manuscript, Salama MA, shared in writing manuscript, Ashoush SE, shared in practical work and writing manuscript as well.

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Explanation of figures

F.1: Stool smear: a- stained with Lugol's iodine showing *G. lamblia* trophozoite (x400), b- stained with Lugol's iodine showing *E. histolytica/E. dispar* cyst (x400), c- stained with trichrome stain showing *G. lamblia* cyst (x1000), d- stained with trichrome showing *E. histolytica/E. dispar* cyst (x1000), e- stained with MZN stain showing *C. parvum* oocyst (x1000).

Fig. 2: Correlation between group and clinical presentation.

Fig. 3: Test strip with positive for *E. histolytica/E. dispar, G. lamblia* & *C. parvum* (crimson, green, red and blue bands).

A B C D E

F.1: Stool smear: a- stained with Lugol's iodine showing G. lamblia trophozoite (x400), b- stained with Lugol's iodine showing E. histolytica/E. dispar cyst (x400), c- stained with trichrome stain showing G. lamblia cyst (x1000), d- stained with trichrome showing E. histolytica/E. dispar cyst (x1000), e- stained with MZL stain showing C. parvum oocyst (x1000).