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Comparative Study Between Guiac and Immunological Fecal Occult Blood (Schebo Test) Techniques for Diagnosis of Fecal Occult Blood in Patients with Intestinal Parasites

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Abstract: *Background*: Intestinal parasitic infection is one of the most infectious diseases in developing countries; unnoticed bleeding which is known as fecal occult blood (FOB) is an important symptom. Colorectal carcinoma, diverticulitis, even swallowing blood due to oral bleeding may result in FOB. Objective: To determine the relation between FOB and intestinal parasitic infection and to highlight on probability of colorectal carcinoma in patients with intestinal parasitic infection. Subjects and Methods: Our study concluded 100 patients with intestinal parasites attending NHTMI were examined for FOB using Guiac and Immunological Fecal Occult Blood tests (GFOBt and IFOBt). *Results*: Twenty patients (20%) were FOB positive by Guiac Fecal Occult Blood test, 39 patients (39%) were FOB positive by Immunological Fecal Occult Blood test. Patients with *Heterophyes heterophyes,* or *Fasciola spp.* showed FOB negativity by both techniques. The highest percent for FOB positivity (72.7%) was in patients with *Ascaris lumbricoides* by IFOBt and in 6 patients (50%) with mixed parasitic infections by GFOBt. "Faecal M2-PK" was positive in 5 patients as: 3 patients with mixed parasitic infection, one case with *Entamoeba histolytica* and one case with *Schistosoma mansoni* infection. *Conclusion/Recommandations*: Our study illustrated a high prevalence of FOB positivity especially by use of IFOBt that was more accurate and needs no prior restrictions before testing. We recommend using of FOBt as a routine screening test in our hospitals especially for those with parasitic diseases and for further investigative studies on a bigger number of patients to determine the magnitude of colorectal carcinoma in intestinal parasitic diseases.

Key words: FOB · FOB Tests · Intestinal Parasites

INTRODUCTION

One of the most prevalent infections in tropical and developing countries is human intestinal parasitic infections (IPs) that cause significant rate of both morbidity and mortality [1]. Hygiene, poor socioeconomic status, overcrowding, food and water contamination play great role as risk factors, infection may be asymptomatic, while one third of the world population is suffering from IPs infections present with clinical manifestation as abdominal pain, nausea, diarrhea, fever or give rise to unnoticed bleeding which is known as fecal occult blood (FOB) [2]. There are other different causes resulting in FOB, as in early stages of gastrointestinal illness as colon

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carcinoma, polyps, colitis, diverticulitis ulcer, or even fissure that may not show visible symptoms, but only present with FOB, sometimes, fecal blood could be from swallowed blood due to oral bleeding [3]. FOB test detection was mainly developed for colon carcinoma screening [4]. FOB (refers to cryptic fecal blood), has been used as diagnostic laboratory tool for intestinal pathological changes specially when associated with colorectal carcinoma [5]. Also, FOB can be used as diagnostic tool for identification of blood loss due to enteric parasitic infection as hookworm, *Trichuris trichura* or even in *Entamoeba histolytica* infections [6]. So, fecal occult blood test is considered as screening tool that may help in diagnosis aiming to most effective treatment in gastrointestinal bleeding. There are many names for FOB test as FOBT, stool occult blood, occult blood, Hemoccult test, Guaiac based smear test, immunochemical FOBT, IFOBT, FIT. Guaiac fecal occult blood test may show lack of sensitivity and specificity, also it requires food stuffs and medical drugs restrictions before testing. Rapid GFOBT is recommended for health professionals in routine physical examinations for monitoring GIT bleeding in hospitalized patients, as a simple visual qualitative chromatography test that can detect human FOB in fecal samples within 5 minutes [2].

Fecal hemoglobin can be detected by a modern immunological fecal occult blood test (IFOBT), which recognizes only human hemoglobin (HB), this is known as ScheBo test, which is visual Immunochromatographic rapid stool test for detection of M2-pk and human hemoglobin (HB) in stool samples. ScheBo test provides improved colorectal cancer screening and can indicate inflammatory bowel disease, digestive tract diseases or human FOB and M2-pk. M2-pyrovate kinase in stool sample is an innovative enzyme biomarker which is completely independent from blood in the stool. Simultaneous testing of M2-pk and HB makes it possible to detect non-bleeding as well as bleeding colorectal polyps and cancer. The test result is not influenced by drugs or food stuffs (so no special diet or drugs are required prior to collecting the stool sample) [7, 8].

Objective: This current study was conducted to study relation between IPs infection and FOB using traditional GFOB test and modern immunological technique.

The study was aiming to detect the most easily, accurate and rapid technique that can be considered as valuable respectable screening test for FOB. The detection of FOB could get over the health hazard of gastrointestinal blood loss that mostly leads to anemia and its complications also to spot light on probability of colorectal carcinoma occurrence in patient infected with gastrointestinal parasites.

MATERIALS AND METHODS

Patient's Group: This current study was conducted on 100 patients attending NHTMRI from April 2019 to February 2020, all subjects proved to be infected with intestinal parasites with no prior drugs / food stuffs restriction before samples collection and investigations. All patients provided verbal or written informed consent.

Sample Collection: Fresh stool sample was collected from each patient in a dry, clean, labeled container. All samples were examined immediately for parasitological and FOB detection.

Laboratory Investigations:

I- Parasitological Examination:

1-Macroscopic examination: The presence of pus, worms, larvae, visible blood and also fecal sample consistency were detected.

2-Microscopic examination: After preparation of the fecal samples by:

- Formol -Ether concentration method [9].
- Direct smear method [10]. For dysenteric and unformed specimens, preparation was done without adding saline, but we placed a drop of eosin reagent [11].

Not to stain living trophozoites, but provides a pink background which can make them easier to see.

II- FOB detection:- (Rapid FOB and Schebo tests):

1-FOB rapid test (GFOBt): - commercially available by CLUNGENE, Hamburg, Germany.

The test is in-vitro, rapid, chromatographic assay for qualitative detection of human blood hemoglobin (HB) in stool samples.

Test Principle / Interpretation: FOB rapid test is a qualitative membrane strip-based immunoassay (Antigen/Antibody reaction). If fecal specimen contains

Table 1: Cross Reactivity and Interference (according to the manufacturer's protocol of test kit)

		Specimens	
Analysts	Conc. (mg/ml)	Positive	Negative
Beef hemoglobin	0.5	+	-
Chicken hemoglobin	0.5	+	-
Pig hemoglobin	0.5	+	-
Goat hemoglobin	0.5	+	-
Horse hemoglobin	0.5	+	-
Rabbit hemoglobin	0.5	+	-
Horse radish peroxidase	2	+	-

hemoglobin antigen, a colored line will appear indicating a positive result. If the specimen does not contain hemoglobin antigen, the line will not appear indicating a negative result.

Test Procedure: FOBT was performed according to the manufacturer's protocol of test kit.

Specimen containing the previous substances at the standard concentration was tested on both HB positive and negative specimens and showed no effects on test results at standards concentration. No cross reactivity or interference was observed to the test kit (Table 1).

ScheBo (M2 - PK + HB) Test: The test is in-vitro immunologic diagnostic test, commercially available by ScheBo. Biotech AG, Germany.

Test Principle: Pyruvate- kinase is a key enzyme in glucose metabolism that exists in various isoforms, its active form consists of four equal subunits (tetramer). With tumor development, the tissue-specific isoforms are lost and replaced by expression of the M2 form of isoenzyme.

ScheBo test (2 × 1) is a Quick stool test based on an Immuno-chromatographic technique used for detection of fecal M2-PK and HB by two monoclonal antibodies which are bound to gold particles. Fecal M2-PK or HB reacts with this respective monoclonal antibody, which is bound to gold particles.

This complex migrates along the membrane to the test line (T) that has a second monoclonal antibody against M2-PK or HB attached.

In positive results, the gold-labeled antibody-M2-PK complex or gold-labeled antibody HB complex binds to the test line (T) with development of pink color.

In negative results, sample does not contain antibody-M2-PK or HB complex that can bind to the test band (T), so no color develops.

A pink control line (C) development confirms that application and migration of fecal sample have taken place correctly.

Interference (according to the manufacturer's protocol of test kit).

- Very watery stool samples can lead to false-negative results due to a dilution effect.

Test Procedure: FOBT was performed according to the manufacturer's protocol of test kit.

Interpretation:

- When negative test result is obtained inspire of the presences of diseases symptoms, further diagnostic investigations should be arranged.
- Positive test result for M2-PK and/or hemoglobin (HB), this can be an indicator of colorectal polyps or colorectal cancer.
- Positive test result for M2-PK can occur in acute and chronic inflammatory bowel disease and other diseases of the digestive tract.
- Positive test result for HB can occur in colorectal polyps, colorectal cancer, gastrointestinal bleeding e.g. parasitic diseases, hemorrhoids, menstrual bleeding, severely bleeding gums or hematuria.

Statistical Analysis: A database was created; all collected data were coded and double entered to ensure high quality of data entry.

Data were analyzed using IBM SPSS statistics version 24.

Qualitative data were expressed as frequency and percentage.

RESULTS

Table 2 shows that, 13% of studied samples were positive for *E. vermicularis* followed by 12% in mixed infection and 11% of samples positive for *Ascaris, H. nana* accounts for 9%, *E. histolytica, while Taenia* and *S. mansoni* each accounts for 8% of cases.

Giardia lamblia, E. coli, Fasciola spp., *S. stercoralis* and *H. hetrophyes* account for 7%, 5% 4%, 4%, 3% respectively.

Table 3 showed that, out of 12 mixed infections, 6 cases were FOB positive by Guaiac test and 7 by immunologic HB "ScheBo" test.

Both tests showed one positive FOB case out of 9 cases of *H. nana* samples.

Table 2: Distribution of study samples (100 samples) according to the type of parasite

Type of Parasite	Percent (%)
Mixed infection	12
Hymenolepis nana	9
Giardia lamblia	7
Enterobius vermicularis	13
Entamoeba histolytica	8
Ascaris lumbricoides	11
Heterophyes hterophyes	3
Taenia spp.	8
Fasciola spp.	4
Ancylostoma duodenale	8
Stronglyoides stercoralis	4
Schistosoma mansoni	8
Entamoeba coli	5
Total number	100

Table 3: Comparison between Guaiac and Immunologic HB (ScheBo) tests in detection of occult blood in the stool of patients with parasitic infection

	Guaiac Test		Immunologic Hb		
	Positive		Positive		Total parasitic infection
Parasite	N	%	N	%	(100%)
Mixed infection	6	50	7	58.3	12
Hymenolepis nana	1	11.1	1	11.1	9
Giardia lamblia	3	42.9	5	71.4	7
Enterobius vermicularis	2	15.4	1	7.7	13
Entamoeba histolytica	--	--	4	50	8
Ascaris lumbricoides	2	18.2	8	72.7	11
Heterophyes heterophyes	--	--	--	--	3
Taenia spp.	--	--	1	12.5	8
Fasciola spp.	--	--	--	--	4
Ancylostoma duodenale	4	50	7	87.5	8
Strongyloides stercoralis	--	--	1	25	4
Schistosoma mansoni	1	12.5	2	25	8
Entamoeba coli	1	20	2	40	5

Table 4: Frequency distribution of cases with positive M2 PK test in relation to positive immunologic HB "ScheBo" test

	Immunologic HB		M2 PK test		
	Positive		Positive		Total parasitic infection
Parasite	N	%	N	%	(100%)
Mixed infection	7	58.3	3	25	12
Hymenolepis nana	1	11.1	--	--	9
Giardia lamblia	5	71.4	--	--	7
Enterobius vermicularis	1	7.7	--	--	13
Entamoeba histolytica	4	50	1	12.5	8
Ascaris lumbricoides	8	72.7	--	--	11
Heterophes hetrophyes	--	--	--	--	3
Taenia spp.	1	12.5	--	--	8
Fasciola spp.	--	--	--	--	4
Ancylostoma duodenale	7	87.5	--	--	8
Strongyloides stercoralis	1	25	--	--	4
Schistosoma mansoni	2	25	1	12.5	8
Entamoeba coli	2	40	--	--	5

Out of 7 cases with *Giardia lamblia* infection, 3 were positive FOB by Guaiac test and 5 by immunologic HB "ScheBo" test.

Out of 13 cases with *E. vermicularis* infection, 2 cases showed occult blood in their stool by Guaiac test versus only one case by immunologic HB "ScheBo" test.

Out of 8 cases with *E. histolytica* infection, 4 samples had positive occult blood by immunologic HB "ScheBo" test.

Out of 11cases with *Ascaris* infection, 2 cases showed occult blood in stool by Guaiac test versus 8 cases by immunologic HB"ScheBo" test.

Out of 8 cases with *E. histolytica* infection, one sample had positive occult blood by immunologic HB "ScheBo" test.

Out of 8 cases with *Ancylostoma* infection, 4 cases showed occult blood in stool by Guaiac test versus 7 cases by immunologic HB "ScheBo" test.

Out of 4 cases with *S. stercoralis* infection, one sample had positive occult blood by immunologic HB "ScheBo" test.

Out of 8 cases infected with *S. mansoni,* one case showed occult blood in stool by Guaiac test versus 2 cases by immunologic HB "ScheBo" test.

Out of 5 cases infected with *E. coli,* one case showed occult blood in stool by Guaiac test versus 2 cases by immunologic HB "ScheBo" test.

No occult blood was detected in cases infected with *Fasciola* or *H. heterophyes* by both tests.

Table 4 showed that, M2 PK test was positive in 3 cases with mixed infection, one case of *E. histolytica* and one case of *S. mansoni*.

DISCUSSION

There are various diseases resulting in positive FOB, including some types of parasitic infections, from the parasitological point of view, our study aimed to detect and determine the correlation between intestinal parasitic infections and positive FOB by using two different FOB tests in some patients infected with IPs infections, attended NHTMRI.

Some authors in previous studies detected that most common parasites causing dysentery and blood loss was *Trichuris trichiura*, hookworms, *Schistosoma mansoni* and *E. histolytic*a [12], others illustrated that FOB presences was not correlated to *Trichuris trichiura* infection, expect in *Trichuris* dysentery syndrome (TDS) [13]. There were four asymptomatic

amebic colitis patients with FOB positive results due to *E.histolytica* infection [6]. Some authors demonstrated that unless there was a high burden of hook worms and there was no significant occult gastrointestinal bleeding [14], it was also reported that anemia can occur in *Schistosoma spp*. infections as result of fecal blood loss in high-burden infection [15].

In 2010, Wakid [12] detected that *T. trichiuria*, hookworm, *S. mansoni* and *E. histolytica* were the most common parasitic infections causing blood loss in stool, while it was proved that this occurs only when accompanied by dysentery syndrome [13]. The current study was conducted on 100 intestinally infected patients, 12 patients (12%) had mixed infection, 9 (9%) *H. nana*, 7 (7%) *G. lamblia*, 13(13%) *E. vermicularis*, 8 (8%) *E. histolytica*, 11 (11%) *Ascaris*, 3 (3%) *H. heterophyes*, 8 (8%) *Taenia spp*, 4 (4%) *Fasciola spp*., 8 (8%) *Ancylostoma*, 4 (4%) *S. stercoralis*, 8 (8%) *S. mansoni* and 5 (5%) *E. coli* infection respectively.

Our results showed that the most common parasites causing blood loss with positive fecal FOB detected by GFOB test were 6 (50%) in mixed parasitic infected patients, 4 (20%) in *Ancylestoma,* 3 (42.9%) in *G. lamblia*, 2 (18.2%) in *E. vermicularis* equally to *Ascaris* respectively, while the most common parasites causing blood loss with positive FOB detected by IFOBt were 8 (72.7) in Ascaris, 7 (87.5%) in *Ancylestoma* equal to mixed infection 7 (58.3%), while it was 5 (71.4%) in *G. lamblia*, 4 (50%) in E. histolytica, 2 (40%) in *E. coli* 2 (25%) in *S. mansoni*. 1 (11.1%) in *H. nana*, 1 (7.7%) in *E. vermicularis*, 1 (13.5%) in *Taenia spp*, 1 (7.5%) in *S. stercoralis*.

Also, our results showed that negative FOB results for cases infected with *H. hetrophyes* or *Fasciola spp.* were detected by both GFOBT and IFOBT, while negative results of FOB were detected by GFOBt in cases infected with *E. histolytica*, *Tenia spp*. and *S. stercoralis*.

Many authors studied the specificity, sensitivity, accuracy and also ability of detection of colorectal carcinoma using different FOB techniques.

In an experimental study on the *Haemonchus contortus* (blood-sucking parasites) in lambs that cause presence of FOB by using three types of FOB tests aiming to demonstrate a new laboratory indicating marker of *Haemonchus contortus* (H.C) infection in sheep, that may be included in genetic evaluation system as alternative selection criterion to fecal egg count (FEC), they proved that FOB test was able to detect H.C infection earlier than FEC [16].

It was stated that GFOB test show positivity of 22.6%, while it was 12.1% by immunoassay FOB test, explaining this by the lack of proper dietary restrictions prior to test, this may lead to incidence of high false-positive results by GFOB test especially in case of having foods containing high level of peroxidase or ascorbic acid (meat, vegetable, fruit) or medication such as aspirin , non-steroidal anti–inflammatory drugs or even in heavy alcohol consumption, that may interfere with the test results [17].

Many authors found that FIT was more specific and more sensitive in the detection in human fecal occult blood and it needs no prior medical or food restriction, also it has predictive values in colorectal carcinoma (CRC) diagnosis, indicating a good performance of the test [3, 7, 18, 19].

In comparative studies between Guiac and immunochemical FOB tests, they detected 50 % and 75 % of sensitivity ratio while the specificity gave 77.87% and 90.12% for GFOB and FIT respectively [8, 20].

It was found that both Guiac and immunologic FOB tests had comparable sensitivity screening detection of proximal and distal colorectal carcinoma, IFOBt showed inferior sensitivity for proximal lesions detection than distal lesions and this relative low sensitivity might affect detection of advanced adenomatous lesions and limit its effectiveness in CRC screening programs [21].

Our results showed that there were 39 patients (39%) from the totally 100 studied patients showed positive results for FOB by IFOBt while there were 20 patients (20%) showed positive results for FOB by GFOBt. FOB in *E. histolytica*, *Taenia spp.* and *S. stercoralis* infections was detected only by IFOBt not by GFOBt.

In a previous study carried out by Lee, *et al.* [22], they demonstrated that positive FOB test could be seen in cases with *E. Vermicularis* infection with colon carcinoma lesion. It was investigated whether FOB could be used as marker for assessment of bowel morbidity that associated in schistosomiasis over time and also after treatment with Praziqauntel, concluding that FOB rapid test could be useful for control Programs aiming to evaluate the extent and dynamics of intestinal morbidity in young children patients at a community level and to monitor changes in morbidity after mass chemotherapy programs [23]. It was stated that FOB tests can be used as a screening laboratory diagnostic test in monitoring of gastrointestinal disorders and evaluation of colorectal carcinoma incidence [24], authors found that FOB by Immunochemical test has an

important role in diagnosis of FOB more than GFOB test in diagnosis of occult gastrointestinal bleeding as it has no need for prior restriction of medications or foods and its more specificity and sensitivity [2, 25]. In a previous study, authors demonstrated that positive FOB can be seen in *E. vermicularis* infection with colon carcinoma [22], while our results obtained by ScheBo test revealed that there were only totally 5 positive cases with M2-PK as: 3 cases with mixed parasitic infection, one case with *E. histolytica* and one case with *S. mansoni* infection, these 5 patients need more investigations by GIT colonoscopy, tumour markers, C.T scan to exclude malignant tumors.

CONCLUSION / RECOMMENDATION

Our study illustrated a high prevalence of positive FOB in intestinal parasitic infections, especially when diagnosed by the use of Immunological HB test.

GFOB test has low-coast, easy to use, can be useful in field screening programs, but it needs prior food and medical restrictions before testing, this can lead to false diagnosis if not be taken in quality control in laboratory procedures, while Immunological HB (ScheBo) test was more accurate with no need to prior restrictions before testing, but it is more expensive, also it has the advantage of M2-PK detection which is laboratory non-invasive technique for colorectal carcinoma that may help in early diagnosis and management, as they have the advantage of high analytical sensitivity and specificity in detection of FOB (human HB) compared to GFOB test.

We recommend using of FOB test as a routine screening laboratory test in our hospitals especially for those with symptoms and signs of parasitic infections at any age and for both males and females, this was also recommended by the American Cancer Society (ACS) and the Center of Medical Service (CMS) of the department of Health of the United States.

We also recommended further research investigation on a bigger number of patients with intestinal parasitic infections to assess incidence of FOB and M2-PK to determine the magnitude of colorectal carcinoma occurrence in intestinal parasitic diseases.

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