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Comparison between Immuno -Chromatography and Ordinary General Stool Examination for Diagnosis Cryptospridium, Giardia and Entamoeba

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Abstract

The aim of the present is to compare between the light microscope examination and antigen detection(EIA) for detection of intestinal parasites in human stool sample.Across-sectional study was conducted by examining of 184 patients stool samples 63 patients of them included (35 females and 28 males)who had admitted to a private laboratory in AL-Nahrwan district /Baghdad city suspected of having a protozoan infection between (May /2020 -till August /2020). Only 63 (34.23%) had detected as a positive cases just two cases of 63 had two kinds of infection , the 63 positive cases distributed as 42 (66.66%) by general stool examination ,rapid test 9 (14.2%) ,the agreement between the two tests in 7 samples (1.111%) and disagreement in 5 (7.93% of samples). Most of infections located at age less than 10 years in both sexes 19 (67.8%) male and 20 (57%) female, then decline for other ages, GSE detect the high positive cases (42/63 ,66.6%), rapid test 9/63 (14.2%),while the agreement and disagreement between the two tests were 7/63 (11.1%) and 5/63 (7.9%) respectively for all ages and both sexes. the highest protozoan infection was for Entamoeba (30/58 , 51.7%) for Giardia and Cryptosporidium were (25/58 , 43%) ,(2/58, 3.4%) respectively, either detected GSE or by rapid test . The sensitivity and specificity for GSE (72.4% ,27.5%) , rapid test (15.5%,84.4%) , the agreement between the two tests (12%, 87.90%).

Keywords: rapid test, Immunochromatography, general stool examination, diarrhea, protozan infection.

1. Introduction

The health of both humans and animals is significantly impacted by parasitic illnesses. Intestinal parasites are one of them, and both in rural and urban regions, they impact a sizable fraction of the global population [1][2]. Although chronic courses of parasitic infections can be asymptomatic or show up in adults as mild, nonspecific symptoms. Since children are more susceptible to parasitic infections and the development of acute symptomatology, this can result in a low clinical index of suspicion for the diagnosis [3][4]. The fecal-oral pathway is utilized to spread the food- and water-borne illnesses brought on by intestinal parasites. They typically have an impact on vulnerable minority groups, such as young children and rural residents in developing nations [5][6][7][8]

The most prevalent intestinal protozoa, according to estimates from the World Health Organization (WHO), are Entamoeba histolytica, Cryptosporidium spp., and Giardia lamblia [9]. The median global burden of disease (GBD) for three parasite species was 0.17 million, 0.5 million, and 2 million DALYs in 2010, respectively. Giardiasis, amoebiasis, and cryptosporidiosis each had median numbers of respectively, 184 million, 104 million, and 64 million cases, all of which are believed to be undercounts. Globally, no deaths from giardiasis were not reported in 2010, although 5450 instances of E. histolytica and Cryptosporidium) [10][11].

Scientific research on the potential It is becoming more and more popular to consider the involvement of intestinal parasites in functional digestive disorders, which are characterized by stomach pain and altered intestinal response to luminal (infectious agents or food) or psychosocial stimuli. Irritable bowel syndrome (IBS), food intolerance, carbohydrate intolerance/malabsorption, dyspepsia, and others are all positively correlated with intestinal protozoal disorders, particularly Giardia intestinalis, according to several authors [12] [13]. Antigen detection (EIAs), light microscopy, and PCR tests are the main three methods used to identify human intestinal protozoan infections. After Dutch microscope pioneer Antony van Leeuwenhoek first described parasitic intestinal protozoa in human faeces in 1681[14]. However, a number of intestinal protozoans cannot be detected using the formalin-ether concentration approach [15].

To improve the precision diagnosis of a tiny parasites, staining procedures were done like, acid fast stain that was used for identification of Cryptosporidium ssp.While in case of E.histolytica cyst need skilled profissional to find it, also sensitive EIA have been develpoed to identify antigens of parasites in feces at the microscopic level. As an illustration, Cryptosporidium species can be found using acid-fast stains. Even skilled laboratory professionals find it difficult, and in the case. of some species (such as E. histolytica based on cyst appearance), impossible, to correctly identify intestinal protozoan infections [16]. Sensitive EIAs have developed to identify antigens in feces that

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are unique to the species of G. intestinalis, E. histolytica, and Cryptosporidium; several of these EIAs are quite sensitive and complement microscopic stool screening in many clinical laboratories [17]. Not all commercially available EIA antigen detection kits are specific for E. histolytica, and some of them lack sensitivity, particularly if fecal samples have been stored for a long [18] Such molecular biological tools are essential for enhancing the precise species identification of a number of intestinal parasites, which are difficult to identify using conventional techniques [19][20].

Giardiasis is characterised by diarrhoea, stomach pain and distension, appetite loss, flatulence, and fast weight loss, in that order. Giardia antigens can be found in the faeces using immunochromatography assays (ICA), which are a simple, accurate, and sensitive alternative to light microscopy. There are currently commercialized immunoenzyme assays (IEAs) that employ monoclonal antibodies to the antigens of parasites and have 99% (99.30% to 100%) specificities and sensitivity ranges of 88.6%-100%. It is debatable whether the serum EIA is helpful in determining the presence of human giardiasis. The specificity and sensitivity are strongly influenced by the kind of antigen used, the immunoglobulin under examination, and the frequency of infection in the area [21][22][23].

The concentration procedures used for the identification of of cyst which then can be diagnosed under light microscope. In addition, trichrome or iron hematoxylin stains can be used for observation of trophozoite or cyst in stained fecal samples. Also, direct immunofluorescence that utilized monoclonal antibodies for antigen identification for precise diagnosis of Giardiasis, the therapy and early managements of illness deepened on it [24][25]. Precise diagnosis. Microscopic analysis of at least three independently collected stool samples is used in the standard laboratory diagnosis to find trophozoites or cysts. The classic diagnosis is base the accuracy of this diagnosis depends on the sampling intervals, patient cooperation, use of concentration techniques, and technologists' knowledge. Additionally, in recent years, various techniques have been used primarily for diagnostic or research purposes, such as molecular techniques and fast immunochromatographic tests (ICTs). Alternative diagnostic techniques that are quicker and more reliable can increase the sensitivity and specificity of all diagnostic techniques [26].

2. Materials and Methods

Study population: (63) patients consisted of (35 female and 28 males) out of 184 stool samples were detected to have one or two of the causative agent suspected of having a protozoan infection, were selected from those who had admitted to a private laboratory in AL-Nahrwan district /Baghdad province laboratory in a period between (5/2020 -8/2020). Fresh diarrheal and non-diarrheal stool samples were collected. General stool examination (GSE)and RIDA® Quick chromatography

test/monolab.kit/Turkey, were done for detection the presence of Cryptosporidium spp., G.lamblia, and E.hitolytica infection, for all cases, GSE done by mixing a tiny part of stool with drop of buffer (Ph=7.2) on a clean glass slide once and with lugols iodine (institute of sera and vaccine/Iraq) other once and covered with cover slip , then examined microscopically under power (X10, X40) . A chromatography test is a qualitative determination of Cryptospridium parvum , Giardia lamblia and /or Entamoeba histolytica in stool sample was done by adding 100 IU(50 mg)of stool to 1ml of buffer diluent homogenize the sample by vortex or by suction and ejection by using disposable pipette (for 3min.), the stool was allowed to settle for 3 min. then 4 drops or 200 UI of supernatant were pipetted into a special well in the cassette .After ten minutes, the results were read. If protozoa are present in the stool, a colored band will be visible across each marker of protozoa in the cassette.

3. Statistical Study

IBM Statistical package for social sciences SPSS (version 20) was used for Statistical study. Also means, standard deviations(SD), Frequency and percentages were included for basic Descriptive. Analytical evaluation of the ratio of GSE and fast test infection rates was conducted using the Chi-square test (x2). When the difference had a P value under 0.05, the difference was considered statistically significant.

4. Results

63 (34.20%) out of 184 stool samples were detected to had one or two of the causative agent ,as shown in Table (1), according to sex ,28(44.4%) were male and 35 (55.6%) were female . most of infections located at age less than 10 years in both sexes , since19 (67.8%) for male and 20 (57%) for female, then decline for the other ages, the GSE detect the high positive cases (42/63 , 66.6%) , rapid test 9/63 (14.2%),while the agreement and disagreement between the two tests were 7/63 (11.1%) and 5/63 (7.9%) respectively for all ages and both sexes.

Table (1): The correlation between age and sex with number of diagnosed cases by Microscope (GSE) and rapid test.

Age(yr.)	Male	Female	GSE	Rapid test	Agreement between(GSE)& rapid test	Disagreement between (GSE) & rapid test
Less than 10 yr.	19(67.80)%	20(57%	25(59.50%)	7(77.70%)	6(85.7%)	3(60%)
11-20yr.	4(14.2%	7(20%)	9(21.2%)	2(22.2%	0%	1(20%
21-30yr.	0%	2(5.7%)	1(2.30%)	0%	1(14.20%)	0%
Above31yr.	2(7.0%)	6(17%)	7(6%)	0%	0%	1(20%)
sutotal	28)44-40%)	3(55.60%)	42(66.60%)	9(14.20%)	7(11.10%)	5(7.90%)

total	63	63(34.20%)

As clear from Table (2) for all ages, the highest protozoan infection was for *Entamoeba* (30/58 , 51.7%) , for *Giardia* and *Cryptosporidium* were (25/58 , 43%) ,(2/58, 3.4%) respectively either detected GSE or by rapid test . the sensitivity calculated as true positive X 100 / total number and the specificity true positive X 100 / total number (after neglecting the disagreement cases) . The sensitivity and specificity will be for GSE (72.40%,27.5%) , rapid test (15.5%,84.40%) and the agreement between the two tests (12%, 87.90%).

Table (2): The distribution of parasitic infection according to the age stages of patients and number of diagnosed cases by Microscope (GSE) and rapid test.

Age(yr.)	GSE	Rapid test	Agreement between GSE+ rapid test	Disagreement betwee GSE & rapid test
	NO.of paasites	NO.of paasites		
<10 yr.				
E. histolytica	15	1	1	3(E….G)**
G.lamblia	9	3	5	
C.spp.	0	2	0	
E+G*	1	1	0	
11-20yr.				
E.histolytica	6	1	/	
G.lamblia	3	0		1(E……G)**
C.spp.	0	1		
E+G*	0	0		
21-30yr.				/
E.histolytica	1	/	0	
G.lamblia	0		1	
C.spp.	0		0	
E+G*	0		0	
Above 30yr.		/	./	1(E…G)**
E.histolytica	4			
G.lamblia	3			
C.spp.	0			
E+G*	0			
subtotal	42	9	7	5
total	58			5

Note : abbreviations *(E) Entamoeba , (G)Giadia , (C) Crypyosporidium*
(*) detect *Entamoeba* + *Giadia* for the same stool sample.
(**) detect Entamoeba by GSE and *Giadia* by rapid method for the same stool sample.

5. Discussion

Three of the most significant parasite protozoa that cause diarrhea include Entamoeba histolytica, Giardia lamblia, and Cryptosporidium spp. Microscopic analysis of stool samples has

long been regarded as the "gold standard" for identifying infections with G. lamblia, C. parvum, and E. histolytica. For all three of these parasite diseases, more accurate and sensitive alternative techniques, such as multiplex real time PCR enzyme-linked immunosorbent test, have recently been developed [27][28].

However, including these parasite-specific procedures for identification of each of the aforementioned illnesses into a normal diagnostic laboratory is time-consuming and raises the price of a stool examination , so as the simple and fast assay is the rapid test which depend in immunochromatography principal for diagnosing the protozoa antigen in fecal specimens . For intestinal parasite infections, age is a significant risk factor. Due to their poor hygiene practices, frequent contact with contaminated soil, and immaturity of their immune systems, children are more susceptible to intestinal infectious diseases than adults. Because they are more susceptible to parasite infections than any other age group, school-aged children are also more at risk for disease than any other age group. Children between the ages of 5 and 14 are thought to carry 12% of the world's burden of intestinal worm illness, since younger children are predisposed to heavy infections with intestinal parasites because of having not fully developed immune systems and also habitually they play in fecal contaminated soil [29][30][31] .

In present study ,the most infections of the three protozoan located at small ages less than 10 years(19/28 67.8%, 20 /35, 57%) for male and female respectively , then decline for the other ages (Table-1) ,younger children are more likely to catch the diseases, and those with compromised immune systems are particularly at risk ,and these in agreement with that reported by Khan who found that the highest rate of infection under 24 months and less among those 48-60 months of age[32][33], Another study in Ethiopia, Jordan and Qatar demonstrated that persons over the age of 14 were less susceptible to infection than youngsters . People under the age of 14 were more susceptible to infection than those above the age of 14 (OR = 2.6, P = 0.038). This can be because the immune system is still developing, people spend more time outdoors, or they are curious and explore their environment without practicing good hygiene. The impact is stronger when the environment is deficient in infrastructure, such as working sewage systems, clean water, and protection from trash exposure due to dirty streets and insufficient dumping sites[34][35] .

Recording a high percentage of infection (63/184,34.2%), may be explained by as in processing nations ,Urban environments that are crowded and have poor sanitation increase the likelihood of transmission.Chi- square analysis revealed high significant difference between the two assays[36] . The agreement between the assays in the present study was7/63(11.1%) ,whileCostache et al.,2009 [37] found an agreement (100%) and this may be attributed to differences in sensitivity

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and specificity of rapid method kits used in each study but even the low agreement between the assays may be a good sign for using the rapid test to strength diagnosis by microscopy since the antigen persistence several days after treatment and that would be of benefit in examining the delayed samples which brought to laboratory and in monitering the clinically status of patient, as well as the sensitivity and specificity of GSE (72.4%,27.5%) , rapid test (15.5%,84.4%)and the disagreement in 5/63(7.9%) could help to keep in mind that the patient might had another infection that missed when the stool sample had been examined microscopically or the influence of one pathogen by therapy than the other.

However, accurate determination of the incidence of these infections is hampered by infrequent testing of stool for protozoa when patients present with gastroenteritis by inappropriate test ordering by physicians and by the lack of sensitive techniques by which to identify pathogenic protozoa in stool specimens [38].

The aim of the present study: Is to compare between the light microscope examination and antigen detection (EIA) for detection of parasitic infection in the stool sample.

6. Conclusions

In conclusion it was clear that in spite the GSE depend on using microscope in examining the fecal samples and this need a good experience and training to distinguish between the pathogen and other food particles which is still “gold standard assay and the rapid method could help in diagnosis even in a small number of cases. Also, Urban environment that are crowded and poor sanitations, had high percentage of infection and the differences in sensitivity and specificity of rapid method kits used in the present study may be a good sign for using rapid test to strength the diagnosis by microscopy, since antigen persistence several days after treatment.

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