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A Comparative Evaluation of ELISA and Microscopy to Diagnose Intestinal Parasitic Infections in Patients of Pediatric Age Group

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Abstract

Background: Intestinal parasitic Infections (IPI'S) are major health problem in most parts of the world; especially in the under developed and developing countries. *Giardia lamblia*, *Entamoeba histolytica*, *Ascaris lumbricoides* and hookworm are commonly encountered parasites. The study was planned to estimate the proportion of IPI's in patients of pediatric age group and do a comparative evaluation of ELISA and Microscopy for Giardia.

Materials and methods: Stool samples were taken from 200 patients of pediatric age group with the given inclusion criteria, during August 2017 to July 2018. Microscopy, formalin ethyl acetate concentration method and ELISA for Giardia was performed.

Results: The prevalence of intestinal parasitic infections in our study was found to be 28%. The most common pathogenic parasite was found to be *Giardia lamblia* (19.5%) followed by *Entamoeba histolytica* (4.5%). In our study, stool samples positive by Microscopy and formalin ethyl acetate concentration were 8% and 15%. ELISA and Rapid kit test were found to detect higher proportion of Giardia as compared to Formalin ethyl acetate concentration method and Microscopy (Saline and Iodine mount).

Conclusion: The prevalence of intestinal parasitic infections in our study was 28%. The most common parasite was *Giardia lamblia* followed by *Entamoeba histolytica*. The study showed that parasitic infections were common in patients of uneducated families. Patients who did not wash their hands with soap and water before meals and after using toilet were at increased risk.

Keywords: ELISA; Intestinal parasitic infections; Pediatrics; ELISA

Introduction

Intestinal parasitic Infections (IPI's) are major health problem in most parts of the world; especially in the under developed and developing countries [1]. IPI'S are estimated to affect around 3.5 billion people globally and 450 million are ill due to these infections, the majority being children [2,3].

The estimation of morbidity and mortality due to intestinal parasitic infections are of major concern [1]. Over 270 million preschool age children and over 600 million school going children live in areas where these parasites are intensively transmitted [4]. The protozoans *Giardia lamblia* and *Entamoeba histolytica* and the helminths *Ascaris lumbricoides*, hookworm, *Hymenolepis nana* and *Strongoloides stercoralis* larvae are commonly encountered parasites. They cause manifestations like iron deficiency anemia, growth retardation in children and other physical and health problems [5]. Helminthic infection is also related to protein energy malnutrition, low pregnancy weight and intra uterine weight loss [6]. Parasitic infections cause detrimental effects on the physical growth of the general population and leads to poor cognitive functions in children [7]. Symptoms presented by the patients usually depend on the host immune system, the degree of malnutrition, and environmental load [8]. The hot and humid climate of tropical and subtropical countries provides the ideal environment for the survival of eggs or larvae of parasites. Open air defecation, poor sanitation, scarcity of portable water and low standards of personal hygiene are a few factors contributing to it [9,10].

Therefore, it is important to access the burden of intestinal parasitic infections in the Indian community. The study was aimed to estimate the proportion of IPI's in patients of pediatric age group and do a comparative evaluation of ELISA and Microscopy for Giardia.

Material and Methods

The Hospital based Observational study was carried out in the Post Graduate Department of Microbiology and Post Graduate department of Pediatrics, King George's Medical University, Lucknow over a period of one year from September 2017 to August 2018. 200 samples from patients less than 12 years of age presenting with any two of the following features-Abdominal pain, Diarrhea (with or without mucus, blood), Fever, Nausea, Vomiting, Perianal itch, Anemia, underweight for age were taken.

Parents/ Guardian of the child submitted the stool sample in a clean, dry leak proof container to the OPD collection center which was properly labelled, (5 gm of solid stool, 10 ml if consistency of stool is liquid) and was transported to the laboratory within 2 hours. One stool sample from each patient was taken. History of patients was taken in the Laboratory.

For each patient following protocol was followed:

1. Microscopy (Saline and iodine mount),
2. Formalin- ethyl acetate concentration method,

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3. Modified Kinyoun's staining,
4. Rapid test for Giardia, Cryptosporidium and Entamoeba,
5. ELISA for Giardia.

Sample storage and processing

Samples were processed immediately for microscopy. An aliquot of about 0.5 ml was refrigerated at -20°C for subsequent ELISA testing. Stool samples were grossly examined for color, consistency, presence of blood or mucus, parasites etc.

Microscopic examination was done (both saline and iodine mount). On samples found negative on microscopy, Formalin-ethyl acetate Sedimentation concentration method was performed.

Wet mount microscopy

2 mg of stool sample was taken on the tip of a wooden applicator stick and mixed with a drop (0.05 ml) of 0.85% NaCl on a microscopic slide. It was covered with a 22- by 22 mm coverslip and scanned under low power objective (10X) to detect eggs, larvae, cysts and trophozoites and then at high power (40X) to detect eggs, larvae, cysts, trophozoites, red blood cells, pus cells, fat globules and fungal elements. A drop of iodine solution was placed at one edge of the coverslip and drawn into the wet mount by placing a piece of filter paper against opposite edge. The slide was viewed with a low power objective (10) to detect eggs, larvae, cysts and trophozoites and then at high power (40X).

Formalin-ethyl acetate sedimentation concentration method

Half teaspoon (about 4 g) of fresh stool was transferred into 10 ml of 10% formalin in a round bottom tube. The stool and formalin were mixed thoroughly and allowed to stand for a minimum of 30 min for fixation. This suspension was strained through wet gauze (no more than two layers of gauze) into a conical 15-ml centrifuge tube to give the desired amount of sediment. The tube was filled with 10% formalin almost to the top of the tube and centrifuged for 10 min at 500x g. The amount of sediment obtained was approximately 0.5 to 1 ml. This step was repeated once again if the supernatant fluid after the first wash was not light tan or clear. The supernatant fluid was discarded, and the sediment was resuspended in 10% formalin. Only half of the tube was filled. 4 to 5 ml of ethyl acetate was added to it. The stopper was put on the tube and shaken vigorously for at least 30 s. It was then centrifuged for 10 min at 500x g. Four layers result after the procedure: a small amount of sediment (containing the parasites) in the bottom of the tube; a layer of formalin; a plug of fecal debris on top of the formalin layer; and a layer of ethyl acetate at the top. The plug of debris was freed by ringing the plug with an applicator stick. The supernatant fluid was decanted and discarded. The sediment was put on the slide. Both iodine and wet mount were prepared by it and examined at 10X and 40X (Practical Medical Microbiology, Mackie and McCartney).

Modified Kinyuon's acid-fast stain (Cold method)

Fecal smear was made with concentrated (500x g for 10 min) sediment of fresh stool and allowed to air dry. It was fixed with absolute methanol for 1 min. The slide was flooded with Kinyoun's carbol fuchsin for 5 min. Then it was rinsed (3 to 5 s) with 50% ethanol followed by rinsing the slide thoroughly with water. It was decolorized with 1% sulfuric acid for 2 min or until no more color runs from the slide. The slide was rinsed with water and counterstained with methylene blue for 1 min. The slide was again rinsed with water, air dried and examined with the low or high dry objective and then 100X (Practical Medical Microbiology, Mackie and McCartney) was done to identify coccidian parasites.

Rapid test for Cryptosporidium/Giardia/Entamoeba

The Rapid test was performed using commercial kit RIDA^{R} QUICK Cryptosporidium/ Giardia/ Entamoeba Combi, R-Biopharm AG, Darmstadt, Germany as per manufacturer's instructions.

ELISA for *Giardia lamblia* antigen

The ELISA was performed by commercial kit- *Giardia lamblia* antigen ELISA, NovaTec Immundiagnostica GmbH, Germany as per manufacturer's instructions.

Results

A total of 200 stool samples were taken. Patients less than 12 years were enrolled out of which 120 (60%) were males. Diarrhea (72.0%) was the chief presenting complaint followed by Altered bowel habits/poor appetite (43.5%) and Fever (37.5%). The most common pathogenic parasite was found to be *G. lamblia* (19.5%) followed by *E. histolytica* (4.5%). Other parasites were *A. lumbricoides* (0.5%), *A. duodenale* (0.5%) and *C. parvum* (1%). The parasitic prevalence in the study population is shown in Table 1.

Comparative evaluation of ELISA, Rapid test and microscopy for *Giardia lamblia* in the study. Table 2 shows ELISA (19.5%) and Rapid kit test (18.5%), were found to detect higher proportion of *Giardia lamblia* as compared to Microscopy (formalin-ethyl acetate) (10%) and Microscopy (Saline and Iodine) (4.5%) (Figures 1-4).

Association of few risk factors was also studied in our study. Table 3 shows that unawareness, weaning less than 6 months and improper hand cleaning were associated with increased risk of developing infections.

Parasite	Microscopy (Saline and wet mount)	Microscopy (Formal ether)	Modified Kinyoun's staining	Rapid kit	Giardia ELISA
Ascaris lumbricoides	1 (0.5)	1 (0.5)	-	-	-
Entamoeba histolytica	1 (0.5)	4 (2.0)	-	9 (4.5)	-
Ankylostoma duodenale	1 (0.5)	1 (0.5)	-	-	-
Enteromonas hominis	1 (0.5)	1 (0.5)	-	-	-
Entamoeba coli	3 (1.5)	3 (1.5)	-	-	-
Giardia lamblia	9 (4.5)	20 (10.0)	-	37 (18.5)	39 (19.5)
Cryptosporidium parvum	-	-	1(0.5)	2 (1.0)	-
*Figures in parenthesis represent percentage					

Table 1: Parasitic prevalence in study population.

Methods ↓	Positive	%
Microscopy (Saline and iodine mount)	9	4.5
Microscopy (Formal ethyl acetate concentration)	20	10.0
Rapid Kit	37	18.5
ELISA	39	19.5

Table 2: Comparison of Microscopy, Rapid test and ELISA for *Giardia lamblia* (N=200).

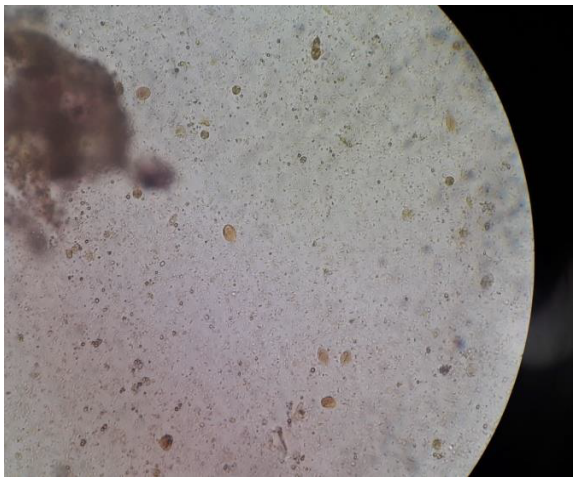

Figure 1: Cysts and trophozoites of Giardia.

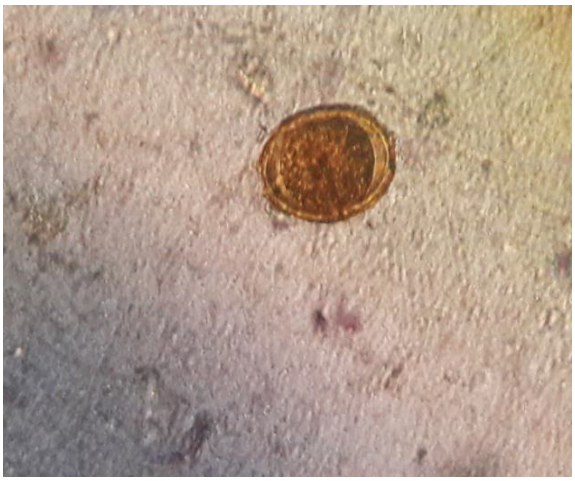

Figure 2: Fertilized egg of *A. lumbricoides*.

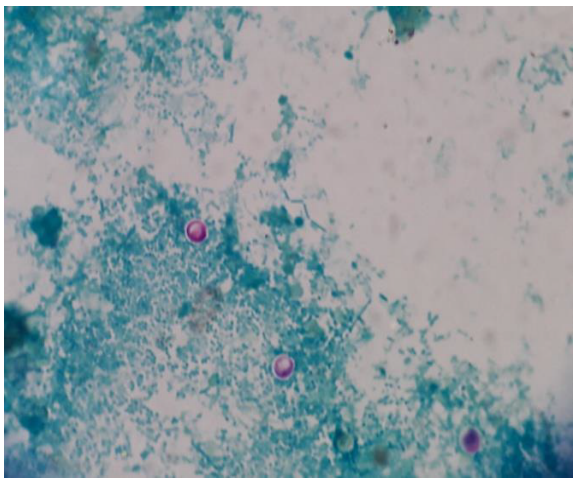

Figure 3: Oocysts of Cryptosporidium spp.

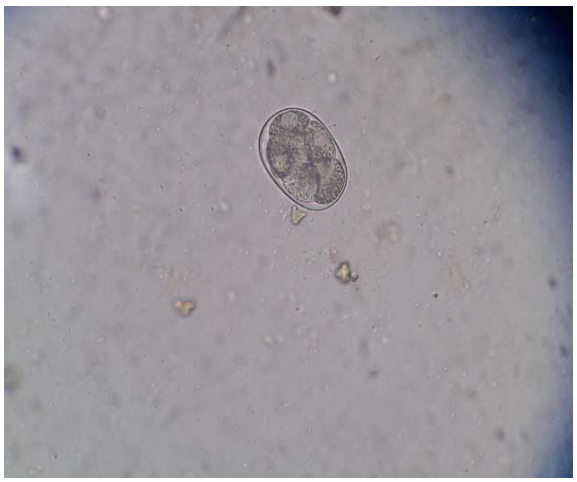

Figure 4: Egg of Hookworm.

S No	Risk factors	Total (N=200)	Infected (n=56)		Non-infected (n=144)		Significance of difference	
			No.	%	No.	%	χ^2	'p'
1-	No/Incomplete. immunization	21	4	7.14	17	11.81	0.933	0.334
2-	Weaning <6 months	81	39	69.64	42	29.17	27.412	<0.001
3-	Uneducated	70	31	55.36	39	27.08	14.168	<0.001
4-	No/Occasional handwash with soap before meals	114	40	71.43	74	51.39	6.606	0.010
5-	Occasional handwash with soap after using toilet	170	55	98.21	118	81.94	9.140	0.003

Table 3: Association of Intestinal Parasitic Infections with Risk factors.

Table 4: Prevalence of intestinal parasites in various studies across the world.

Study	Prevalance
Sangwan J, et al. [14]	17.5%
Sneka P, et al. [15]	24.09%
Kumudavathi, Sandhya Bhat, et al.	55%
Saurabh, et al. [1]	19%
Bisht, et al.	38%
Purbey, et al. [16]	11%
Hailegebriel, et al. [9]	65.5%
Taheri, et al.	47.7%
Iwunze JI, et al.	53.5%
This study	28%

Discussion

IPI's are major health problem in many parts of the world [1]. Open air defecation, poor hygiene and sanitation, scarcity of portable water are a few factors contributing to it. Microscopy is being widely used for diagnosis of intestinal protozoa [11,12]. But nowadays molecular diagnostic tests are increasingly being used for both clinical as well as research purposes. These identify a specific antigen or DNA of parasite in stool or serum samples through ELISA [13].

The prevalence of intestinal parasites in our study was 28%. Prevalence varies in different regions shown in Table 4. The most common parasite was found to be *G. lamblia* (19.5%) followed by *E. histolytica* (4.5%). Other parasites found were *A. lumbricoides* (0.5%), *A. duodenale* (0.5%) and *C. parvum* (1%). Similar prevalence was shown by studies done by Singh et al. [10] and Jyoti Sangwan et al. [14].

ELISA (19.5%) and Rapid kit test (18.5%) were found to detect higher proportion of *Giardia lamblia* pathogens as compared to Microscopy (Formalin-ethyl acetate concentration method) (10%) and Microscopy (Saline and Iodine) (4.5%).

Males (60%) were more commonly affected in our study. This is in accordance with other studies done by Sneka et al., Purbey et al., Parameshwarappa et al. and Champa et al. [15-18]. Our study reports patients aged between 5-12 years were more affected which is like studies done by Sneka et al. and Purbey et al. [15,16].

Majority of the patients enrolled in our study belonged to Urban areas (72.0%). The increasing trend of infection in urban areas could be due to increase in overcrowding. Urban migration has led to the creation of urban squatter settlements with high polyparasitism rate. Studies on urban ecology emphasize the risk factors for polyparasitism in such settings [19-21].

Majority of the patients enrolled in our study were in early winter (29.5%) and summer (26%). The hot and humid climate provides an ideal environment for survival of eggs and larvae of parasites. However, it has been seen that infection rates are high in unsanitary conditions in colder climates also.

Diarrhea (72.0%) was the chief presenting complaint followed by Altered bowel habits and Fever. In our study, the risk factors that were statistically significant were educational qualification, weaning less than 6 months and improper hand washing habits.

Conclusion

IPI's are a major health problem in the world. The prevalence of IPI's in our study was 28%. The most common parasite was *Giardia lamblia* followed by *Entamoeba histolytica*. The study showed that parasitic infections were common in patients of uneducated families. Patients who did not wash their hands with soap and water before meals and after using toilet were at increased risk.

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