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Original Article

Comparison of Routine and Concentration Techniques on Microscopic Examination of Stool for Parasitic Ova & Cysts

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Abstract:
Objective: To compare the routine and concentration techniques on microscopic examination of stool for parasitic ova and cysts to emphasize importance of concentration technique
Methods: A descriptive study of 100 symptomatic and asymptomatic patients who attended the Microbiology Lab, Pathology Department, Family Hospital, Rawalpindi a teaching hospital affiliated with Rawalpindi Medical College were studied for six months for stool examination. Three samples from each case at three different days were selected not necessarily consecutive. Three techniques were applied on all samples which included direct microscopic examination by using saline and iodine preparations and the microscopic examination after using two concentration techniques which included simple salt flotation technique and Formal-ether concentration technique.
Results: In routine technique 4% of patients were positive for ova/cysts. In saturated saline floatation technique 8% of patients were positive for ova/cysts of parasites. In formal ether sedimentation technique also 8%of patients were positive.
Conclusion: Concentration techniques (formal-ether sedimentation and saturated salt floatation) significantly improved the sensitivity from 4% to 8% for detection of ova/cysts of parasites as compared to routine iodine preparation technique. Although concentration techniques are well known, these need to be implemented in those laboratories where these are not used.
Keywords: Stool Concentration techniques, Parasites, Ova, Cyst, Flotation, Sedimentation technique

Introduction

In developing countries intestinal parasitic infection is one of the serious health problems and approximately 10% of the population is infested with intestinal worms.[1] The continuous presence of intestinal parasites in malnourished children can cause severe anemia and consequently affect the growth and development of these children. Intestinal parasitic diseases are still a significant cause of morbidity in these countries and hence warrant prompt diagnosis and treatment.[2]

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Human intestinal parasitic infestations have a worldwide distribution, with the greatest frequency and concentration occurring in developing countries.[3]The parasites most commonly affect the gastrointestinal tract during their lifecycle.[4] These most common parasitic diseases include amebiasis, ascariasis, hookworm infestation and trichuriasis.[5] Commonly used methods for the detection of intestinal parasite for stool examination are direct and concentration techniques.[6] Concentration techniques are considered to be better as they are supposed to increase the yield and the positivity rate. The main purpose of this technique is to isolate parasites and ova from fecal debris.[7]

The stool concentration techniques are however cumbersome and in many laboratories in developing countries these are not routinely performed. In order to confirm their advantage over simple direct examination we carried out this study. Our study

reaffirms the advantage and hence need for their implementation in all laboratories.

Material and Methods

100 patients including 71 males, both symptomatic (51% had anemia) and asymptomatic, were studied for 6 months. This descriptive prospective study was conducted at Pathology Department, Rawalpindi Medical College, Rawalpindi. For stool examination three samples from each case at three different days; not necessarily consecutive, were selected.

The inclusion criteria included all outdoor patients of any age, both genders who were sent for stool examination by treating physicians. The exclusion criteria included history of ingestion of tetracyclines, sulfonamides, antiprotozoal agents, laxatives, antacids, castor oil, magnesium hydroxide, barium sulphate, bismuth kaolin compounds and hypertonic salts 1-2 weeks prior to the sample collection.

Fresh stool sample was collected in a proper sized clean plastic container with a tight fitting lid. The samples were protected from contamination with water, soil and urine. A portion of the specimen was processed immediately at the site for direct iodine preparation examination using diluted iodine following standard procedure [8]. (Figure-1).

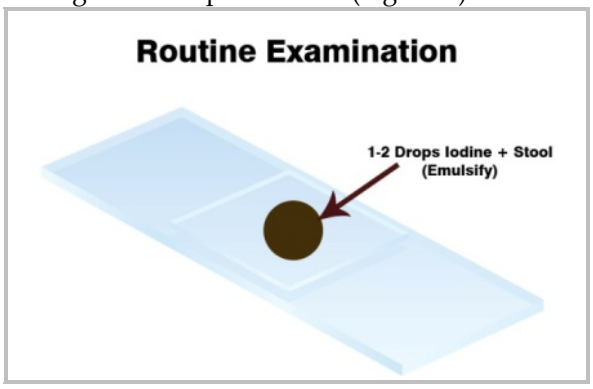

Figure 1: Direct examination method

The remaining sample was processed for concentration techniques i.e. simple salt flotation technique and formal-ether concentration technique. In simple salt flotation method, about 1gm of feces were emulsified with 3-4 ml of saturated salt solution in a 20ml conical glass test tube. After stirring well, more salt solution was added till the container was nearly full, with the stirring being continued. Any extra floating coarse matter was removed and the tube was placed on a leveled surface with a glass slide being placed over the top of the tube, which was in contact with the fluid. It was allowed to stand for 30 minutes. The slide was removed and observed for the presence of eggs/cysts at magnification of 100 and 400. (Figure 2)

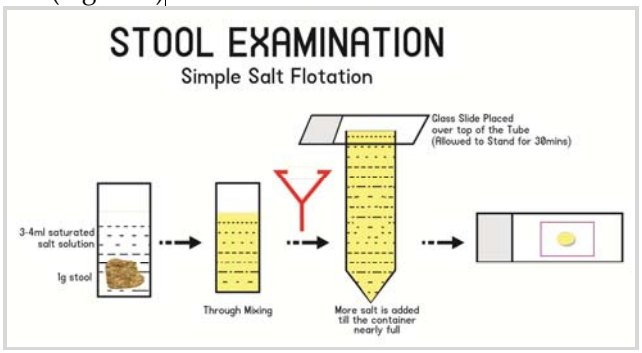

Figure 2: Salt flotation technique

In formal ether sedimentation technique, one gram of stool was emulsified in 7ml of 10% formal saline and it was kept for 10 minutes for fixation. It was then strained through wire gauze. The filtrate was added to 3 ml of ether and it was centrifuged at 3000 rpm for 1 minute. It was allowed to settle. The supernatant was removed and a wet mount was made of the deposit to look for parasites at magnification of 100 and 400. [8]All stool samples were processed by all three techniques of stool examination (routine, sedimentation and floatation technique). (Figure 3)

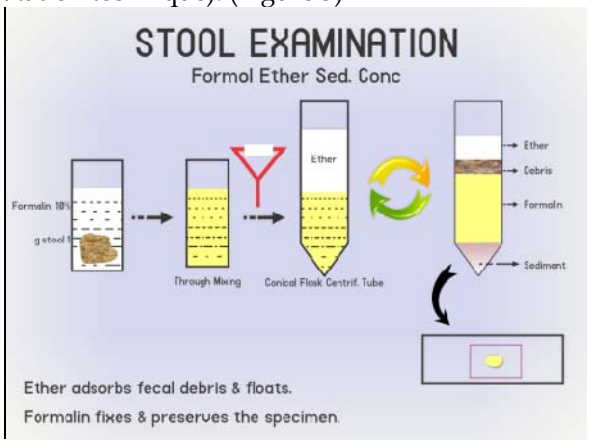

Figure 3 Formal Ether Sedimentation Method

Results

In routine technique 4% of patients were positive for ova/cysts. Ascaris lumbricoides eggs were seen in 3% and H. nana in 1% cases. In formal ether sedimentation technique 8%of patients was positive for ova/cysts. Ascaris lumbricoides eggs were seen in 2%,H. nana ova in 3%,Taenia saginata in 2% and ova of Ancylostoma duodenale in 1% cases in were seen. In saturated saline floatation technique 8%of patients

were positive for ova/cysts of parasites. These included 3% ova of A. lumbricoides, 3% ova of H. nana, 1% cyst of Giardia lamblia and 1% ova of Enterobius vermicularis. (Table 1).

Gender	Ova/cysts Not seen (%)			Ova of Ascaris lumbricoides seen (%)			Ova of Hymenolepis nana seen (%)			Ova of Taenia saginata seen (%)			Ova of Ancylostoma duodenale seen (%)			Cysts of Giardia lamblia seen (%)			Ova of Enterobius vermicularis (%)			Total		
	RT	ST	FT	RT	ST	FT	RT	ST	FT	RT	ST	FT	RT	ST	FT	RT	ST	FT	RT	ST	FT	RT	ST	FT
Male	68	64	65	2	1	2	1	3	3	0	2	0	0	1	0	0	0	1	0	0	0	71	71	71
Female	28	28	27	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	29	29	29
TOTAL	96	92	92	3	2	3	1	3	3	0	2	0	0	1	0	0	0	1	0	0	1	100	100	100

Table 1: Positive stool samples for ova/cysts by three techniques in gender of patients

RT= Routine technique, ST= Formal ether technique, FT= Saturated saline floatation technique

Discussion

In developing countries like Pakistan, parasitic infestations are an important cause of morbidity and mortality. The data on their prevalence and the sensitivity of various diagnostic methods help the clinicians and the microbiologists to diagnosis and manage patients with parasitic infestations. Various studies have shown different prevalence rates of the parasitic infestations in different parts of the world. Most of the studies had compared two techniques[9]. We however compared routine direct techniquewith formal ether sedimentation and saturated saline floatation techniques. Various studies have shown varying sex prevalence of the parasitic infestations, however, the sex predominance for the parasite infections has still not been confirmed. The reason for high incidence of parasitic infestation in the male population in our study may be related to the daily activity of males rather than the sex predominance.[10]

The diagnosis of parasitic infections in humans is challenging and it requires skills to identify and differentiate them from one another. There is low sensitivity in the routine diagnostic procedures. Concentration allows the detection of the organisms which are present in small numbers and may be missed by using direct wet mounts. The organisms that can generally be identified by using concentration procedures include: helminth eggs and larvae; cysts of *Giardia lamblia*, *Entamoeba histolytica*, *Hymenolepis nana*. The present study showed that there was a significant increase in the number of parasites which were detected by concentration methods. The inclusion of two or three different concentration techniques with different principles into the routine diagnostic tests increased the sensitivity.[11]

In this study sensitivity of both formal ether method and saturated saline floatation techniques was twice that of routine iodine preparation (8% versus 4%). This result agrees favorably with others similar studies. The intestinal parasites recovery rate for formal-ether was 65.26% and 34.74% for direct smear methods showing the superiority of the formal-ether technique over direct iodine preparation method.[12]

Out of the two concentration techniques one study performed in India in 2014 showed that the total prevalence of intestinal parasites by formal ether sedimentation technique was 27% while flotation technique prevalence was 17.64%. So they concluded that sedimentation technique is easily performed and the chances of technical errors were less as well. They have more sensitivity as compared to flotation technique. Also the morphology of parasitic eggs and protozoal cysts is preserved in sedimentation technique.[13]

In our study different parasites were seen in different techniques. This may be because of the fact that the routine technique may not be sufficiently sensitive as compared to the sedimentation and flotation technique. Various factors may also affect the result. One of them is eggs may not be evenly distributed throughout the feces. However to increase the maximum yield of results both concentration techniques should be applied on the same sample So that the sensitivity may be improved from 8% to 12%. Higher detection rate with this method has been explained by the fact that, by using saturated salt solution of specific gravity of 1:20, which is heavier than that of eggs and cysts, the eggs and cysts float to the top and easier to recover.

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D. Manuscript Writing
E. Critical Review
F. Facilitated for Reagents/Material/Analysis