Original Research Article

Role of concentration techniques and methylene blue wet mount for detection of gastroentestinal parasitic infection: a study from tertiary care hospital

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Abstract

Background:- Parasitic infections remain major public health issue in the developing countries. Most of the laboratories are unable to detect parasitic infections by using routine methods like wet mount due to their compromised sensitivity and specificity. Therefore, demonstration of parasitic infections of the specimen poses a huge challenge to the clinical microbiologists.**Methods:-** A prospective study was conducted over the period of 11 months from August 2018 to June 2019. A total of 200 freshly passed stool samples were transported to the Department of Microbiology in sterile containers.Patients with suspicion of parasitic gastrointestinal infection were included in the study. Fecal samples were immediately examined by iodine, normal saline and Methylene blue wet mounts and then processed by saturated salt solution and Zinc sulfate centrifugation methods, then observed again by with iodine, normal saline and methylene blue wet mounts.**Results:-** A total of 200 stool samples were examined, out of which 64 (32%) samples were positive for gastrointestinal parasites with wet mount. After concentration methods this increased upto 98 (49%). So with different concentration techniques we were able to detect 34 more parasites. These both concentration techniques have the best impact on detection of parasites, but as per our study Zinc sulfate centrifugation method has more diagnostic value to detect gastrointestinal parasites. **Conclusion:-**There is still a need to use reliable, economical diagnostic method which can accurately detect parasitic infections and control its spread.

Keywords: Gastrointestinal parasitic infections, Methylene blue wet mount, saturated salt solution method, Zinc sulfate centrifugation

Introduction

The incidence of human intestinal parasitic infections is high in developing countries. According to WHO, in developing countries out of 3 million people around 450 billion people get infected by intestinal parasites[1,2].In developed countries protozoan parasites most frequently cause gastrointestinal infections in comparison with helminthes. In endemic countries, mortality and morbidity are significantly caused by intestinal parasites[3].Various intestinal protozoan parasites have been reported from the different area of the world such as *Entamoeba histolytica, Cryptosporidium parvaum,Isospora belli, Giardia lamblia, Microsporidia, Blastocystis hominis, Dientamoeba fragilis, Balantidium coli* and *Cyclospora cayetanensis*[4]. Out of these dominant protozoan parasites of worldwide public health apprehension are *Giardia, Entamoeba* and Cryptosporidium. The common geohelminths and soil-transmitted helminthic parasites are *Ascaris lumbricoides* (roundworm), *Trichiuris trichiuria* (whipworm), *H.nana* and *Necator americanicus* (hookworm)[5]. While most parasitic infections are likely to be asymptomatic, and some commens symptoms are dysentery, vomiting and abdominal discomfort [6].The conventional method used for examination of stool is wet mount preparation. It requires a good skill for ideal analysis as it is labor-intensive but for the diagnosis of intestinal protozoa this test remains as a keystone.[7] Parasite detection in stool sample is improved by the use of concentration techniques. Several concentration techniques like simple salt floatation, Zinc sulfate centrifugal floatation are used for the detection and epidemiologic surveillance of parasitic protozoan cyst in fecal specimens. These concentration techniques increase the detection rate of the protozoan cysts[8]. Recent reports have indicated that concentration techniques showed variable efficiency in the detection of intestinal parasites in stool samples[4,9]. Therefore aim of our study is to compare the concentration floatation techniques like saturated salt solution and Zinc sulfate solutions with simple iodine wet mount and methylene blue wet mount(background stain) for gastrointestinal parasitic infection in humans.

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Material and methods

This cross-sectional study was conducted during the period of August 2018 to June 2019. A total of 200 stool samples were collected from patients suspected with parasitic infection and sent to laboratory of Muzaffarnagar Medical College,Muzaffarnagar. Out of 200, 92 were males and 108 were females. Each fecal sample was immediately examined by different wet mounts and processed by both concentration methods. Iodine wet mount and normal saline wet mount were prepared by standard protocol of parasitology laboratory.

1% Methylene blue Wet Mount Technique : With the help of applicator stick, a small quantity (2mg) of stool sample was taken and to it a drop of 1% Methylene blue was added on a clean glass slide. After that, cover slip avoiding the bubbles was put on it and observe under the high power field**.**

Floatation techniques

Saturated salt solution method: About 15- 20 gm of stool sample was taken in a beaker and mixed with 10-20ml normal saline. Extra fecal debris was removed and filtered through gauze. It was then centrifuged at 2500 rpm for 10 min.Sediment was mixed with

saturated salt solution (approx. 36gm%) in a test tube with stirring being continued and the tube was filled till the meniscus was formed. Any floated course matter was removed and a coverslip was placed on meniscus. It was allowed to stand for 30 minutes and then coverslip removed and observed for the presence of ova/cysts.

Zinc sulfate centrifugation method: About 15- 20 gm. of stool sample was taken in a beaker and mixed with 10-20ml normal saline.Extra fecal debris was removed and filtered through gauze. Centrifuged at 2500 rpm for 10 min. The supernatant was discarded and then sediment was re-suspended in normal saline. This step was repeated till the supernatant became clear. To the sediment, 3-4 ml of 33% Zinc sulfate solution was added. The tube was placed on a flattened surface with a coverslip been placed over the top of the tube, which was in contact with the fluid. It was allowed to stand for 30 minutes. The coverslip was removed and observed for the presence of cysts.

RESULT

In the present study, we have collected total of 200 stool samples from patients of suspected with parasitic infection.Out of 200, 92 were male and 108 were female.In the study 64 (32%) samples were positive for parasitic infection by wet mount. After using different concentration methods the positivity rate has increased to 83 (41.5%) with saturated salt solution (NaCl) and with the help of Zinc sulfate centrifugation technique rate of positivity was 98 (49%). **(Table:-1)** So with the help of concentration technique we were able to detect 34 more positive samples. The detection rate of parasitic infections was 49% in this study,in which we have identified *Entameoba histolytica/dispar* (19.5%) followed by *Giardia lamblia* (11.5%), *Ancylostoma duodenale* (4%), *Cryptosporidium* (3.5%), *H.nana* (3%), *Ascaris lumbricoides* (3%) then *Balantidium coli* (1.5%) as shown in **(Table:-2)**

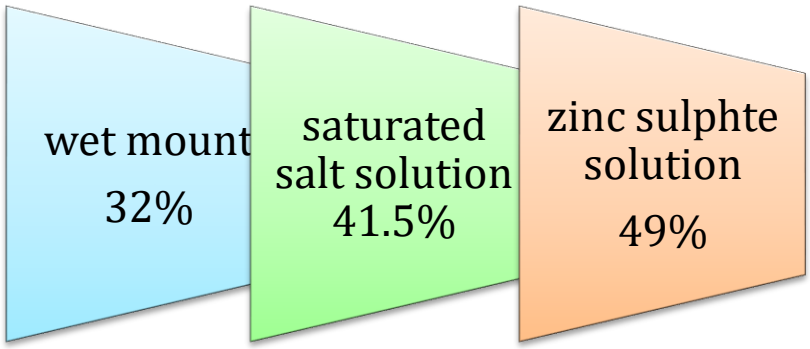


Fig 1:Sensitivity of different parasitic examination technique

Table 1 : Parasite detected in the study

Name of parasite	Number of (+ve) case (N=200)	Percentage
1. Cyst of *Entamoeba histolytica/ dispar*	**39**	19.5%
2. Cyst of *Giardia lamblia*	**29**	11.5%
3.Cyst of *Cryptosporidium*	**07**	3.5%
4. Cyst of *Balantidium coli*	**3**	1.5%
5. Ova of *H.nana*	**06**	3%
6. Ova of *Taenia species*	**04**	2%
7. Ova of *Ancylostoma duodenale*	**08**	4%
8. Ova of *Ascaris lumbricoides*	**06**	3%
Total	**98**	49%

According to this study, males (56.52%) were more frequently found positive with parasitic infection in the comparison to female (42.59%) and detection rate of parasitic infections is higher in 41-50 years age group (74.42%) and followed by 6-10 age groups (65.38%) than <5year age groups (52.27%) and the minimum detection rate in 31-40 age group (35.89%)**.**With the help of Wet mounts we detected 25 *Entameoba histolytica/dispar,* 16 *Giardia lamblia,* 4 *Cryptosporidium, 4 H.nana, 4 Taenia species, 6 Ancylostoma duodenale* and 4 *Ascaris lumbricoides* and with the help of Zinc sulfate centrifugation method we detected 39 *Entameoba histolytica/dispar,* 29 *Giardia lamblia,* 7 *Cryptosporidium, 6 H.nana, 0 Taenia species,8 Ancylostoma duodenale* and 6 *Ascaris lumbricoides***.**

Table 2: Gender wise detection of parasitic infection

Gender	No. of specimen	No. of test positive	%
Male	**92**	**52**	**56.52**
Female	**108**	**46**	**42.59**
Total	**200**	**98**	**49**

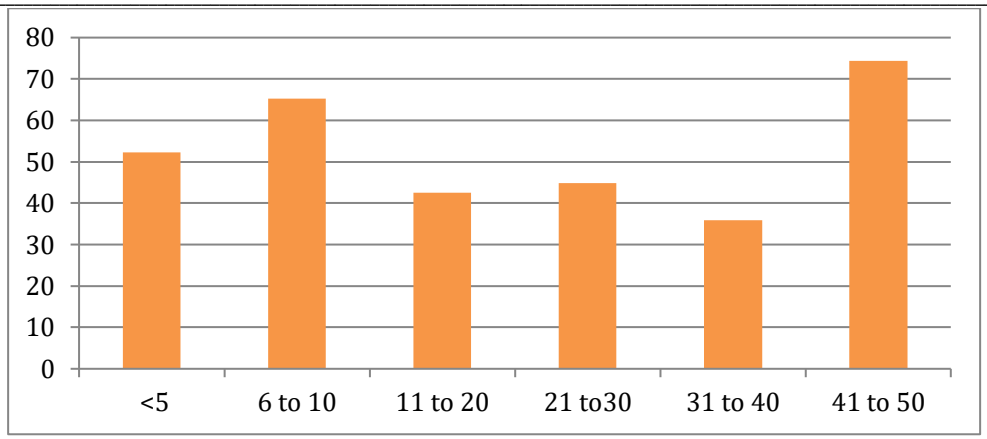
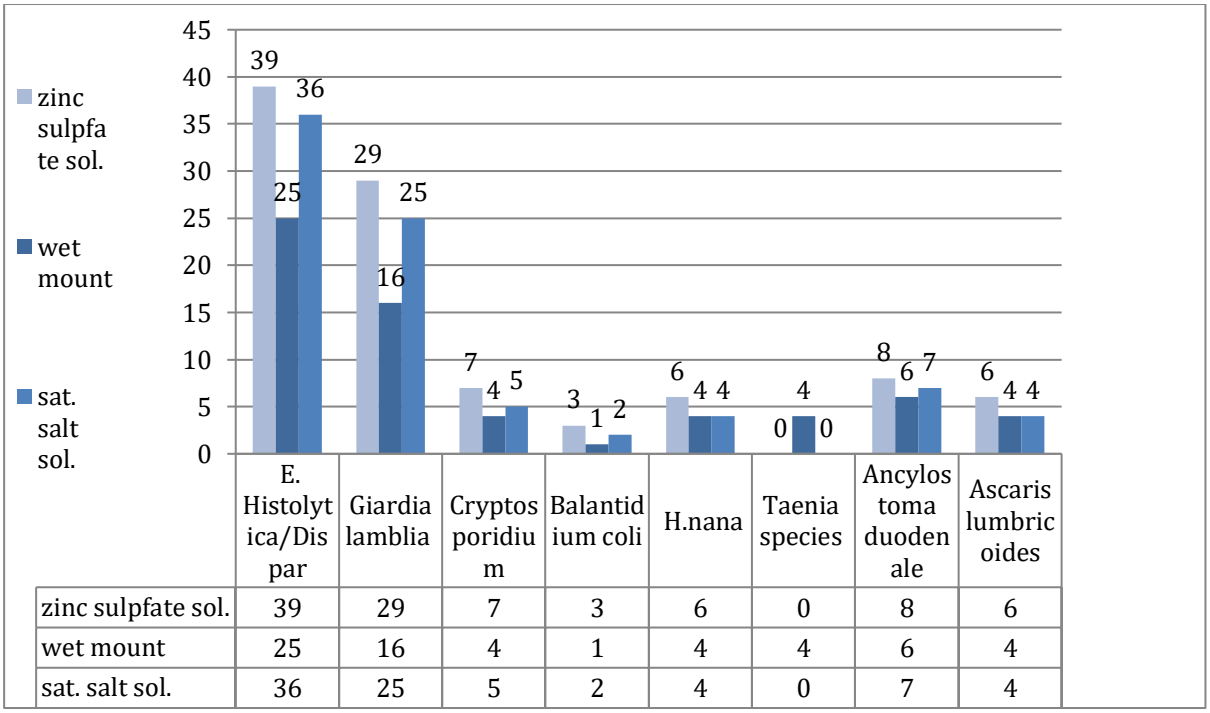


Fig 2: Parasitic Detection in different age groups



	E. Histolytica/Dispar	Giardia lamblia	Cryptosporidium	Balantidium coli	H.nana	Taenia species	Ancylostoma duodenale	Ascaris lumbricoides
zinc sulpfate sol.	39	29	7	3	6	0	8	6
wet mount	25	16	4	1	4	4	6	4
sat. salt sol.	36	25	5	2	4	0	7	4

Fig 3:Rate of parasitic cyst and ova identification by different concentration of stool.

P value= .01 for wet mount and zinc sulfate solution methods

P value .4 also significant to Sat. salt soltion.

Discussion

Intestinal parasitic infections are common in developing countries due to low personal hygienic lifestyle, exposure to animal feces, unpurified water supply in rural areas, no health education in rural areas and yet there is no effective vaccine against the intestinal parasite. Various studies show the different prevalence rate in different parts of India. The detection rate of parasite in our study is 49% and another study was conducted by Bineshlal *et al*.(2009)[10] , in Vellore shows 34% prevalence rate and another study done by Garg.R and Singh V at Mullana (2017) found 45% prevalence rate[11]. The prevalence of parasite is different from area to area and season to season. The most common parasite identified in our study was *Entameoba histolytica/dispar* (19.5%) then followed by *Giardia lamblia* (11.5%), *Ancylostoma duodenale* (4%), *Cryptosporidium* (3.5%), *H.nana* (3%), *Ascaris lumbricoides* (3%) then *Balantidium coli* (1.5%). Other study done by Parameshwarappa KD et al,2012 shows that common organism was *E.histolytica/dispar* (65%) followed by *Ascaris lumricoides* (12%),*Giardia lamblia* (8%),*Hook worm* (8%), H.nana (1.08%),then Taenia spp. (1.81%) and *Enterobious vermicularis* (0.36%)[12]. However education on personal hygiene, environmental sanitation, water supply and treatment play major role in intestinal parasitic infection prevalence. In our study males (56.52%) are more commonly found positive for parasitic infection than the female (42%). May be because in rural areas males are mostly working as farmer or labours and most women work as housewife. They mostly work bare handed or without shoes. So they are more prone to get infected. In our study the most

infections prone age group was 41-50 followed by 6- 10 years age group then <5 then 21-40 then 11-20 and the minimum parasitic load was found at 31 -40 years age group. A study conducted by Venkatesh B.M et.al (2016)[13] shows that age group of 0-5 years patients had the more positive rate followed by 6-10 age group then 11-20 and the minimum infected age group was 40 years and above. Indian government has initiated the program Indian Deworming program in 2005. By this program Indian government provide Albendazole to school children 1 to 19 years old. This is the major factor in decreasing the number of *helminth*ic intestinal parasite and this program also play a major role in decreasing the number of helminthic parasites in younger age and the clinicians broadly used the metronidazole in protozoan infection. Metronidazole is very effective in protozoan infection and most frequently use in abdomen distress. The main concern is detection of parasite in stool in developing countries where they have limited resources. Identification of parasites by the wet mount is requires good skilled microbiologist and time. These wet mount procedures lack sensitivity. So these concentration methods should be routinely used in the laboratory like simple salt floatation, Zinc sulfate centrifugal flotation. By these methods we can enhance the identification rate of parasite in stool. In our study, we have used different floatation techniques which show zinc sulfate solution floatation technique was more sensitive to identify parasite (49%) than saturated sodium chloride method (41.5%) where in wet mount preparation, we were able to demonstrate only (34%).So the use of different concentration techniques for identification of parasite shows more sensitivity and we were able to detect 34 more parasites with the help of a Zinc sulfate centrifugation method. Wet mounts was only able to detect 25 *Entameoba histolytica/dispar,*16 *Giardia lamblia,* 4 *Cryptosporidium, 4 H.nana, 4 Taenia species, 6 Ancylostoma duodenale* and 4 *Ascaris lumbricoides* then samples proceed with Zinc sulfate centrifugation method we detected 39 *Entameoba histolytica/dispar,* 29 *Giardia lamblia,* 7 *Cryptosporidium, 6 H.nana, 0 Taenia species*,*8 Ancylostoma duodenale* and 6 *Ascaris lumbricoides* parasites. So, according to this study we can say that zinc sulphate floatation technique should be used in every laboratory of developing countries which have high burden of intestinal parasite. Zinc sulphate should be prepared freshly for optimum result.Iodine wet mount has some disadvantages like it reacts with starch give blue color and reacts with glycogen gives thick brown color and potassium iodide form crystals in wet mount.With normal saline wet mount we cannot differentiate the intracellular organelles. 1% Methylene blue wet mount gives the blue background to the sample. In blue background intracellular organelle is easy to observe and adding one drop of glycerin in Methylene wet mount one can prevent the drying of wet mount.

Conclusion

Intestinal parasite is very cruel and can cause a variety of complications if not diagnosed earliest. So the detection of the ova/cyst can be increased by concentration technique and can prevent the complications. Zinc sulphate technique shows the highest rate of detection of intestinal parasite. Stool artifact reacts with iodine and change the colour, methylene blue can be used and its very effective stain to see parasites in stool. We however still need a method which can detect the intestinal parasite with great sensitivity.

Conflict of Interest: Nil

Source of support: Nil

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