e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(12); 5-8

Original Research Article

To Assess the Diagnostic Accuracy of Wet Mount and Concentration Methods of Stool Examination for Detecting Intestinal Parasite Infections at a Hospital with Tertiary Care in India

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Received: 25-09-2023 / Revised: 23-10-2023 / Accepted: 28-11-2023
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Conflict of interest: Nil

Abstract:

Background and Objectives: To assess the diagnostic accuracy of wet mount and concentration methods in detecting intestinal parasite infections during stool testing.

Methodology: A total of 260 patients provided stool specimens, which were collected and evaluated in the Laboratory of the Department of Microbiology at RIMS, Ranchi during a period of 1 year. The study participants were chosen by the utilisation of a systematic random selection methodology. The stool sample was analysed using the Wet Mount (WM) method and various Concentration Techniques. The data was inputted into Microsoft Excel spreadsheet 2007 and analysed using SPSS version 20.0. The sensitivity, specificity, positive predictive value, as well as negative predictive value were assessed using the combined result as the gold standard. The Kappa value was calculated to assess the concordance of the diagnostic procedures.

Results: The prevalence of females was higher than that of males in both techniques. Through the routine technique, the prevalence was determined to be 17.07% in females and 15.16% in males. Through the Concentration Method, the percentage was determined to be 30.00% in females and 28.85% in males. The age group with the greatest occurrence rate was 6-10 years, namely 66.03%.

Conclusion: The wet mount approach resulted in an underestimation of the frequency of intestinal parasites. Based on these results, we see that the supplementation of concentration method along with the routine wet mount method is more sensitive than the routine wet method alone. Therefore, the WM and various concentration techniques should be used as a routine diagnostic technique for the diagnosis of intestinal parasites identification.

Keywords: Diagnostic performance, wet mount, concentration techniques, Intestinal Parasitic infections

Introduction

Intestinal parasitic infections continue to be a significant public health concern in poorer nations, mostly due to insufficient sanitation and inadequate personal hygiene. It is a prevalent health issue among those belonging to the lower socio-economic strata. Approximately 500 million individuals worldwide may be infected with E. histolytica, resulting in many tens of thousands of deaths annually due to severe colitis or amoebic liver abscess [1]. The estimated number of individuals afflicted by Roundworm, Hookworm, and Whipworm in the developing world is 700 million, 800 million, and 500 million, respectively. Approximately 200 million individuals are afflicted with Giardia lamblia, whereas E. histolytica affects approximately 10% of the worldwide populace [2].

The occurrence of intestinal parasite infections varies based on geographical locations and is also influenced by the diagnostic techniques employed in different laboratories. The Wet Mount Method, which consists of the Normal Saline Wet Mount and Iodine Wet Mount, is the most often employed technique for detecting parasites in the laboratory. This method is easy to perform, low cost and time saving but the sensitivity is low which affects the treatment adversely. Other methods of stool examination are Stool Concentration Techniques .These techniques can detect parasites which are present in small number and can be missed very easily. The present study was conducted with an aim to detect the ova and cyst in the Stool by Direct Wet

Mount method and various Concentration Techniques.

Aim and objectives:

The primary aim of the study was to assess the diagnostic accuracy of wet mount and concentration techniques in detecting intestinal parasite infections by stool testing.

Material and Methods:

A total of 260 patients' stool specimens were collected and studied during a one-year period at the Laboratory of the Department of Microbiology at RIMS, Ranchi. The specimens were gathered in a sterile, dry wide-mouthed container. During the specimen collection process, relevant patient information including age, gender, residence, occupation, income, religion, and education was documented. Details on sanitation facilities, previous occurrences of gastrointestinal sickness, eating patterns, and footwear usage were also recorded. The Macroscopic examination of stool for the colour, consistency, bloody – mucus discharge and the presence of any worms and proglottid or segment of worm was done .

Under microscopic examination, each stool specimen was analysed using the following techniques:

1. Wet Mount Method by:

a) Saline preparation

b) Iodine preparation.

2. Concentration techniques such as:

a) Simple salt floatation.

b) Zinc sulphate centrifugal floatation.

c) Formol-ether concentration.

Wet Mount Method:

In the wet mount method, fresh stool samples (1-2 mg of stool) were emulsified with a drop of physiological saline (0.85%) in Saline preparation. The iodine preparation was created by mixing 1-2 mgs of faeces with 1-2 drops of iodine, resulting in an emulsion. Subsequently, a cover plate was placed over them and they were scrutinised using a microscope, initially employing 10x objectives and subsequently 40x objectives. The mobile form of protozoan parasites was detected in a normal saline sample, but the dormant form was detected in an iodine sample.

Concentration Techniques:

a) Simple Salt Floatation- A 20ml container was used to collect approximately 1ml of excrement. To create a uniform emulsion, a little amount of saturated salt solution was added and mixed with a stick. Subsequently, additional salt solution was introduced until the container reached near maximum capacity, with continuous stirring maintained throughout the procedure. Subsequently, the flask was positioned on a flat and even surface. The last portion was added using a dropper until a raised curved surface was created. A glass slide was delicately positioned above the container. After a 30-minute incubation period, the glass slide was carefully inverted to prevent any liquid from leaking. A cover slip was positioned on top of it and inspected using the microscope.

b) Zinc-Sulphate Centrifugal Floatation – To create a high-quality mixture of faeces, 1 gm of faeces was combined with 10 millilitres of lukewarm distilled water. The larger particles were eliminated by the process of filtration. The liquid that passed through the filter was gathered into a tube designed for spinning and spun for 1 minute at a speed of 2,500 revolutions per minute. The liquid portion above the sediment was eliminated, and purified water was introduced. The mixture was vigorously agitated, subjected to centrifugation, and this procedure was repeated 2-3 times until the liquid above the sediment became transparent. Next, the last liquid portion was removed and 3-4ml of a solution containing 33% Zinc sulphate was introduced. The sediment was agitated, and the tube was subsequently filled with Zinc sulphate solution until it reached the brim. Subsequently, it underwent another round of centrifugation for a minimum duration of 1 minute at a speed of 2,500 revolutions per minute (r.p.m.). Then, the surface layer was extracted using a platinum wire loop and transferred onto a pristine glass slide. A cover slip was then placed on top, and the specimen was then analysed.

c) Formol – Ether Concentration Technique - A 10% formalin solution was used to emulsify one gm of excrement, which was then kept for 10 minutes to undergo fixation. The mixture was subsequently filtered using a wire mesh and the resulting liquid was collected in a centrifuge tube. A volume of 3 millilitres of ethyl acetate was introduced into it, and the resulting combination was forcefully agitated for duration of one minute. The sample was subjected to centrifugation at a speed of 2,000 revolutions per minute for duration of 2 minutes, followed by a period of allowing the components to separate. The liquid portion above the sediment was poured out, leaving behind a little amount of the solid material. The deposit was agitated and transferred onto a glass slide. A cover slip was positioned on top of it and the specimen was scrutinised.

Observation & Results:

The prevalence was determined to be 41 (15.76%) using the Routine Method and 64 (29.22%) using the Concentration Method (Table-1). The study found that the total prevalence was 40.38%. Kaliappan et al [3] conducted a research in a tribal area of

Southern India and determined that the overall prevalence rate was 39%.Taenia had the greatest prevalence rate at 21.15%, while A lumbricoides had the lowest prevalence rate. The other parasites found are E. histolytica (10.76%), Hookworm (7.30%), G. intestinalis (5.76%) and H.nana (2.69%).

The prevalence of females was higher than that of males in both techniques. Through the routine technique, the prevalence was determined to be 17.07% in females and 15.16% in males. The Concentration Method revealed a female prevalence of 30.00% and a male prevalence of 28.85%. The age group with the greatest occurrence rate was 6-10 years, namely 66.03%.

Table-1: Displaying the total prevalence of intestinal parasitic infections by the use of routine examination and concentration methods

Stool samples received from RIMS	Method	Total no. of samples	Total no. of positive samples	Percentage
	Routine	260	41	15.76%
	Concentration	219	64	29.22%

Table-2: Presenting the total prevalence of intestinal parasitic infections by the use of routine examination and concentration methods.

	Protozoa		Helminthes			
METHOD	E.histolytica	G.intestinalis	Taenia	H.nana	A.lumbricoides	Hook-worm
Routine	5 (1.92%)	2 (0.76%)	33 (12.69%)	2 (0.76%)	1 (0.38%)	5 (1.92%)
Concentration	23 (10.50%)	13 (5.93%)	22 (10.04%)	5 (2.28%)	3 (1.36%)	14 (6.39%)
Total	28 (10.76%)	15 (5.76%)	55 (21.15%)	7 (2.69%)	4 (1.53%)	19 (7.30%)

Discussion:

Intestinal parasitic infections have been a major problem of morbidity and mortality in developing countries. Though usually not life threatening, chronic parasitic infestation can impair the nutrition, immunity and general health .This can also make the person susceptible for the infections with other pathogen. Morbidity due to intestinal parasites has always been an important public health problem in the tropics, but the incidence and severity may vary depending on the location and period of time [4] (Sethi et al, 1999); One of the reasons could be due to the various methods of stool examination methods which are many a times not be sensitive enough for the recovery of parasites.

Wet Mount Method is commonly used in a routine basis in most of the laboratory for the intestinal parasites as this is a quick and easy method to perform. But the downside of this method is the lack of sensitivity .It fails to recover the parasites in the specimens with low concentration.

Therefore, it is necessary to use a diagnostic procedure that is more sensitive and trustworthy in order to achieve the highest possible recovery of the parasites.

The current study found that the prevalence of Intestinal parasite infections is 15.76% when using the regular wet mount method and 29.22% when using the concentration method. The total prevalence stands at 40.38%. The study conducted by Kaliappan et al [3] in a tribal region of Southern India revealed an overall prevalence rate of 39%. Prevalence rates of the condition in different regions of India have been documented in various studies. In Chandigarh, the prevalence ranges from 7.5% to 15.5% [2] (Sethi et al, 2000), while in Delhi it is reported to be 16.8% [5] (Abraham et al, 1969). In Andhra Pradesh, the prevalence is found to be 47.1% [6] (Das et al, 1981a), while in Tamil Nadu it is 73.4% [7] (Ganga and Ravichandran, 1995). In Gujarat, two studies report prevalence rates of 70.8% and 60% [8] (Das et al, 1981b).

The highest prevalence rate, at 21.15%, was seen for Taenia.In contrast to these findings, previous investigations done by Saxena et al [9] in 1982 and Rao et al [10] in 1971 reported that Taenia had the lowest prevalence. This discrepancy might be attributed to variations in location and examination methodologies employed. Upon comparing several methods of stool inspection, it was shown that the flotation approach was unable to identify the taenia egg. In a separate research conducted by Kang et al [11], the most prevalent parasitic infection was hookworm, followed by Giardia and Cryptosporidium. Table 2 demonstrates that the various concentration procedures outperformed the regular examination for detecting E.histolytica, G. intestinalis, H.nana, A. lumbricoides, and Hookworm, with the exception of Taenia, for which the normal wet mount method was more successful. Vidyarthi et al (1976) also observed the comparable advantage of the routine procedure compared to the Zinc floatation method.

Conclusion:

In conclusion, this study indicates that there is a need of proper diagnostic method for the stool examination to increase the recovery of parasites .The result shows that the detection of parasites is enhanced by the concentration method. Based on these results, we see that the supplementation of concentration method along with the routine wet mount method is more sensitive than the routine wet method alone.

Funding: Nil

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