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A Comparative Study of Concentration Techniques for Detection of Intestinal Parasitic Infections – to Evaluate the Prevalence And to Identify A Better Method of Concentration Technique At A Tribal Tertiary Care Hospital

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Abstract: *Parasitic infection caused by helminths and protozoa are the major cause of human disease in most countries of tropical region. It is estimated that about 3.5 billion people are infected with intestinal parasites of whom 450 million are ill. The prevalence of intestinal parasitic infection varies not only geographically but also in different region in the same country.*

The present study is undertaken to determine the rate of prevalence of intestinal parasitic infection among all age group of people in this tribal area and to determine the best concentration technique to identify maximum number of intestinal parasites.

Material & Methods: *A total of 836 stools samples were collected from patients with symptoms of parasitic infestation and the same were subjected to (1) Gross examination (2) Direct microscopic examination by using saline and iodine preparation and (3) modified Ziehl Neelson staining after fixing with methanol (4) different concentration technique viz.. (a) Brine concentration flotation technique (b) Zinc sulphate flotation technique (c) Formal ether concentration sedimentation technique (d) Merthiolate iodine formalin sedimentation technique.*

Results: *Out of total 836 stools samples the detection rate by various methods was 286(34.2%) for ova and cysts of protozoa , coccidian and helminths. Compared to females (29.75%), males (38%) were more affected.*

The direct smear saline mount could only detect 36% while the maximum detection 66% of intestinal parasite was after Formalin ether concentrated sedimentation technique.

Among the intestinal protozoa, Entamoeba histolytica (53.49%) was the most common followed by Giardia cysts (8.04%) . Coccidian parasites like cryptosporidium parvum and isospora beli were detected in the HIV infected patient only.

Ascaris lumbricoides (16.43%) followed by ancylostoma duodenele (9.09%) were the common helminthic infections.

Conclusion: *Early and prompt diagnosis of intestinal parasitic infection is important as in addition to morbidity and mortality they contribute to malnutrition, growth retardation and diminished work capacity.*

I. Introduction

Parasitic infections caused by intestinal helminths and protozoans account for significant burden of human disease load in developing countries. It is estimated that around 3.5 billion people harbour intestinal parasites and 450 millions are ill as a result of these infections[1]. Poor sanitation, scarcity of potable drinking water and low standard of personal hygiene contributes to rapid spread of the infection[2]. The prevalence of different parasitic disease depends upon environmental , social and economic factors[3]. In India, malnutrition, improper disposal of sewage and non availability of potable water supplies especially in rural and tribal areas are responsible for the high rate of intestinal parasitic infections[4]. The WHO reported that Entamoeba histolytica causes approximately 50 million cases and 1 lakh deaths annually[5]. The number of people who are affected by Giardia lamblia, whip worm, round worm and hook worm in developing world has been estimated to be 200, 500, 700, 800 millions respectively[6].

Due to low density of parasites in the faeces, direct wet mount method can miss parasites (ova, cysts and larvae) and the detection can be enhanced through using concentration techniques. The present study was conducted with an aim to determine the prevalence of intestinal parasitic infection in a tribal tertiary care hospital and to compare the stool concentration techniques for detection and identification of intestinal parasites.

II. Material And Methods

The present study was conducted in the Department of Microbiology, Rajiv Gandhi Institute of Medical Sciences Adilabad for a period of one year. A total of 836 stool samples were collected from patient attending out patients Department and admitted in wards with symptoms of intestinal infestation like diarrhea, vomiting, abdominal pain and weight loss and they were included for the study.

Specimen Collection

Freshly voided stool samples were obtained from these patients in sterile screw capped wide mouthed disposable plastic containers. Care was taken not to include samples which were contaminated with urine and also from patients already on medication. The samples were then transported to the Microbiology Laboratory immediately.

In The Laboratory

The specimen was subjected to

(1) Macroscopic examination was done and the color, consistency nature presence of mucus and blood were noted and also observation was made for presence of adult helminthic worms with the help of hand lens.

(2) Microscopic examination was done using direct preparation of (saline and iodine wet mount) for detection of trophozoites and cysts of protozoa and ova of helminths.

Modified Ziehl Neelson technique was done after methanol fixation for the specimens suspected to have been taken from HIV Patients for detection of coccidian parasites like cryptosporidium and isospora.

Concentration Techniques Performed

(1). Brine concentration flotation technique : saturated solution of NaCl (brine solution) is prepared and a small amount of faeces is mixed with 2ml of brine solution in a bijou bottle. More brine solutions added till the brim of bijou bottle while stirring. Drops of brim solution are added to the surface of bottle without over spilling. A clean glass slide is placed over the solution surface and left for 30 minutes exactly. The slide is lifted in single hand motion and examined under Microscope.

(2). Zinc sulphate centrifugal floatation technique : 1g of the stool specimen was emulsified in 10 parts of tap water and it was strained through a wire gauze. The filtrate was collected in a Wassermann tube and centrifuged at 2500 rpm. The supernatant was discarded and sediment was re- suspended in water. This step was repeated till the supernatant became clear. To the sediment, 3 to 4 ml of 33% Zinc Sulphate solution was added, it was mixed well and it was filled with $ZnSO_4$ solution, up to about half an inch of the rim. Several loop full of the supernatant fluid was removed with a bacterial logical loop and they were observed for parasites.

(3). Formol – ether concentration sedimentation technique (Allen and Ridely modification) : 1g of stool was emulsified in 7ml of 10% formol saline and it was kept for 10 min for fixation. It was then strained through a wire gauze. The filtrate was added to 3 ml of ether and it was centrifuged at 3000 rpm for 60 seconds and allowed to settle. The supernatant was removed and a wet mount was made of the deposit to look for parasites.

(4). Merthiolate –iodine formalin concentration (MIFC) method : The following solution were prepared weekly and stored in amber coloured bottles ready for use. Solution 'A' consisted of tincture metrhiolate 200 ml, distilled water 200 ml, 40% formaldehyde 25 ml and glycerine 5 ml. Solution 'B' consisted of iodine crystals 5 gm, potassium iodide 10 gm and distilled water 100 ml. For each stool specimen, two tubes are prepared - one containing 9.4 ml of solution 'A' and other 0.6 ml of solution 'B', the contents were mixed together immediately before adding to approximately 1 gm of faeces, after the stool specimen emulsified thoroughly. The tube was stoppered and allowed to stand overnight. The contents are mixed again and filtered through surgical gauze the next morning. Ethyl ether is added and tube is shaken vigorously. After keeping the tube for 1 min, centrifugation is carried out at 1800 rpm for 2 min. Four zones are formed with this technique, the faecal plug is separated and the upper three zones decanted. The sediment is thoroughly mixed and a drop was placed on a slide covered with a coverslip, and examined.

III. Results

A total of 836 stool samples were examined, out of which 286 (34.2%) samples were positive for intestinal parasitic infestation, as was observed by the different parasitic diagnostic methods.

Overall, the prevalence of parastic infections in males and females was 38% and 29.75% respectively (table-2). Children who were up to 0-5years of age(60%) had the highest prevalence of the parasitic infestations(table-3) .

The most common intestinal protozoa cyst isolated was Entamoeba hystolytica (53.49%) followed by Giardia cysts (8.04%) and the helminthic eggs isolated was Ascaris lumbricoides eggs (16.43%) followed by Hook worm eggs (9.09%) (table-1)

Dual infections were seen in 45/286 patients. The most common dual infection was the infestation of the Entameba histolytica cysts with Ascaris eggs.

Table 1: Prevalence of parasitic infestation

Parasite	**Total no isolated**	**%**
Entamoeba hystolytica trophozoites and cysts	153	53.49
Giardia cysts	23	8.04
Entamoeba coli	8	2.79
Cryptosporidium	6	2.09
Isospora	3	1.04
Ascarislumbricoides eggs	47	16.43
Hookworm eggs	26	9.09
Hymenolepis nana eggs	7	2.44
Trichuris trichura eggs	6	2.09
Enterobius vermiculars	7	2.44
TOTAL	**286**	

Table 2: Showing Sex prevalence of parasitic infection

Sex	**No of cases**	**Positive**	**%**
Male	436	167	38%
Female	400	119	29.75%

Table 3: Showing age wise prevalence of parasitic infections

Age	**No of cases**	**Positive**	**%**
0-5yrs	178	107	60
6-10yrs	39	17	43
11-20yrs	161	59	36
21-30yrs	284	68	24
31-40yrs	153	29	19
40and above	21	6	28
Total	836	286	34

Table 4: Sensitivity of different parasitic examination methods

Procedure	**No positive for parasites (286)**	**%**
Direct smear (Saline and iodine mount)	102	36
Brine concentration flotation technique	125	44
Zinc sulphate centrifugal floatation	157	56
Formol – ether concentration sedimentation(allen and Ridely modification)	188	66
Methiolate–Iodine Formalin sedimentation Concentration (MIFC) method	183	64

The usually followed diagnostic method in the laboratory is the saline/ Iodine wet mount which could demonstrate only poorly with a sensitivity of 36% (102/286).

In this study the most sensitive concenterated method was found to be the Formol – ether concentration sedimentation(allen and Ridely modification) with a sensitivity of 66% (188/286) (table-4)

IV. Discussion

Intestinal parasitic infections rank among the most significant causes of morbidity and mortality in the world[7].In the present study the protozoan intestinal parasite Entamoeba histolytica followed by Giardia lamblia were the most prevalent species. This is in agreement to study made by Harsh Ahmed Amin and Shahnaz Abdul Khadar Ali[8].This is also in agreement to study made by Parameshwarappa and Chandrakanth[9] whose study also reported a prevalence of about 65% of Entamoeba histolytica among the isolates of intestinal parasite.Entamoeba histohytica is responsible for approximately 50 million cases of invasive amoebiasis and about more than 1 lakh deaths annually (WHO 1997). This may be due to their chlorine resistant status and its frequent contamination with food and water (Petri and Singh 1999). The next intestinal parasite of increased prevalance that was isolated was the helminth ascaris lumbri coides (16.43%). This finding is comparable to the results of Parameshwarappa et al and Marothi Y. et al[10] and Bishh D et al[11] who also reported similar incidence in their study[12]. Several studies have demonstrated a high prevalence of intestinal helminthic infections in under-

privileged community[13]. It is estimated that 25% of world population are infected with ascaris lumbricoides and this causes up to a million cases of deaths annually[13]. Intestinal helminthic infections are common in poor socio-economical status in tropical and sub tropical region because of poverty,over crowding poor environmental sanitation and low level of education[13].The frequency of parasitic infections differs with age and sex of general population. Intestinal parasitic infections are more common in children[14]. The prevalence rate of parasitic infection was higher in males (38%) as compared to females (29.75%) . In a similar study by Parameshwarappa et al[9] has reported a prevalence rate of (33.39%) in males and (21.29%) in females.

The reason for male preponderance in our study may be related to daily activity rather than the sex preponderance. However sex predominance for parasitic infections is still not confirmed. Children under 5 years of age (60%) has the highest prevalence of parasitic infection. This is an agreement to study conducted by Harsh Ahmed Amin et al[8] who reported (81%). Similar prevalence rates were reported by Al-Kubaisy et al[15] and Farhan et al[16] which showed the infection declined progressively with age.The prevalence in all age groups is high and it may be attributed to poor personnel hygiene and environmental exposure in this tribal area.

The diagnosis of parasitic infections in humans is challenging and it requires skill to identify. Routine diagnostic methods such as wet mount lack sensitivity. Concentration methods must therefore be adopted for increasing the sensitivity for identification of intestinal parasites in stool.

In the present study, a comparison has been made between different concentration techniques along with direct wet mount and it was found that there is a significant increase in the number of parasites after application of concentration techniques.

As per our study, formal ether concentration sedimentation (Allen & Ridely modification) was more sensitive (66%), followed by MIFC (64%), Zinc Sulphate centrifugal flotation (56%), brine concentration flotation technique (44%) and the lowest detection was by wet mount technique (36%). A similar study by Dr. Jai Shree Puri et al[12] reported prevalence of intestinal parasites by formal ether concentration technique was 26.75% while it was 17.64% by Zinc Suplhate. A similar study by Moges F et al[17]reported formal ether concentration technique as more sensitive compared to the other methods. Another study by Parameshwarappa et al[9] reported formal ether method to be the most sensitive method with 64.5% detection.

However, in a study by Hersh Ahmed Amin et al[8], the sensitivity of Zinc Suplhate flotation was found to be 49.3% while formal ethly sedimentation concentration yielded a sensitivity of 43.3%.

As per our study, direct wet mount was less sensitive (36%) when compared to concentration method. However, it had the advantage of being able to provide a quick diagnosis of a heavily infected specimen.

The concentration of stool allows detection of parasites even though in small numbers in stool where the direct smear fails to reveal any parasite. The present study showed that there is a significant increase in the number of parasites which were detected after the concentration techniques were applied.

Thus the formal ether concentration technique is recommended as it is easier to be perform, allows recovery of the broadest range of organisms and is least subject to technical error[18.] It must be included in stool examination detection of intestinal parasites, especially in rural and tribal setup as it is cost-effective and requires minimum basic infrastructure.

V. Conclusion

Intestinal parasites are world-wide in distribution and their prevalence in rural and tribal areas is high due to illiteracy, lack of personal hygiene, lack of access to potable water, poor sanitation. The climatic condition favor the development and survival of parasites and some of the factors contribute to high level of intestinal parasitic transmission.

Early and prompt diagnosis of intestinal parasitic infections is important as this contributes to malnutrition, growth retardation and diminished work capacity, in addition to morbidity and mortality. The formal ether sedimentation concentrated technique along with merthiolate iodine formalin sedimentation technique have high sensitivity. This can be supplemented by Zinc Suplhate flotation concentration technique and can be adopted in the laborataries to increase the diagnostic sensitivity.

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