Detection of cryptosporidium in fecal sample by three staining method from immunocompromised patients

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Abstract

Introduction: Cryptosporidium species is the most common opportunistic enteric parasite encountered in the immunocompromised patients.

Objective: To evaluate Modified Ziehl-Neelsen staining, auramine phenol staining and safranine methylene blue for the diagnosis of intestinal cryptosporidiosis.

Materials and Methods: A total of 70 faecal samples from HIV seropositive were collected. They were subjected for modified ZN stain, safraninemethylene blue stain and auramine phenol stain for detection of cryptosporidium.

Results: Out of total 70 HIV patients, 57 (81%) were positive for parasitic infection, higher number of parasitic infection is caused by Cryptosporidia in 34(60%). Of the 34 patients who had cryptosporidiosis were positive for auramine phenol technique, 33(97%) of them were also positive with the modified ZN stain and 31(91.0%) were also positive with the safranine methylene blue stain.

Conclusion: Auramine phenol staining is a rapid, sensitive and specific technique for diagnosis of intestinal cryptosporidiosis.

Keywords: Cryptosporidium, Modified ZN stain, Safranine methylene blue stain, Auramine phenol stain.

Introduction

Cryptosporidium is an obligated intracellular protozoan parasite that has been identified as one of the major causes of enterocolitis and waterborne diarrheal disease in human. Food and water are the main sources of infection in humans.[1] Cryptosporidia causes acute, severe, self-limited disease in immune competent individuals. In immunocompromised individuals, they cause a severe, intractable, sometimes fatal diarrhea.[2] Prevalence varies seasonally and geographically.This is a worldwide parasite and it has been reported from different parts of the world. In children and adults with diarrhea or other gastrointestinal symptoms, cryptosporidia are often the most common parasites recovered and are frequently the most common enteric pathogens recovered[2]. Cryptosporidiosis should be considered a major cause of diarrheal disease, but the Cryptosporidium oocysts are not easily recognized from other similar artifacts using routine staining procedures.Conventional methods for identification include examination of fecal smears or concentrates using acid-fast stains, safranine methylene blue stain or auramine-phenol stains. These methods require an experienced microscopist to identify the organisms.

Materials and Methods

The study was carried out at Sheth V. S General Hospital, Ahmedabad which is tertiary care Centre. Stool samples were collected from 70 HIV positive cases, who were admitted in the hospital within the period of August 2013 to September 2016.These patients were already confirmed for HIV infection as per strategy III of National AIDS Control Organization guideline.

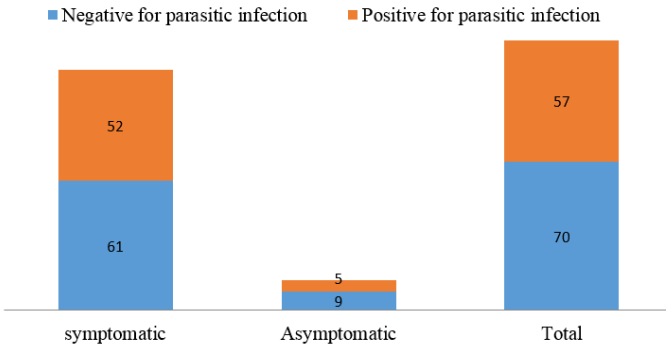
Stool specimens were collected in clean wide mouth, leak proof, plastic containers from each patient. Direct smears of stool samples were prepared and stained by modified acid fast stain, safranine methylene blue method and auramine-phenol stain technique which were used for coccidian.

In modified ZN stain, strong carbolfuchsin, 1% H_2SO_4, methylene blue were used as primary stain, decolorizer and counter stain respectively. 1% Safranin was used for staining and counterstaining was done by methylene blue in safraninemethylene blue staining and slides were examined with 100X objective. For auramine phenol stain, we used Phenolic auramine solution, decolouriser 3% acid methanol and potassium permanganate as counter stain.

In modified ZN stain, oocysts of Cryptosporidium were stained as light pink to bright red, while in safranin methylene blue method, oocysts of Cryptosporidium were stained as a bright reddish orange colour. Oocysts of Cryptosporidium, were seen as ring or doughnut-shaped and fluorescent greenish - yellow against a dark black background in auramine phenol stain.

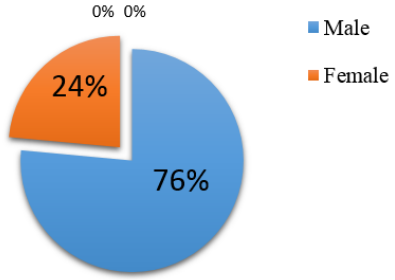
Results

Chart 1: Distribution of intestinal parasitic infection in HIV patients



Out of total 70HIV seropositive patients, 61(87%) were Symptomatic and 9(13%) were asymptomatic cases. Among those symptomatic cases, 52 (91%) were positive for parasitic infection. While out of total 9 asymptomatic cases, 5(9%) had parasitic infection.

Chart 2: Gender distribution in cryptosporidium infection



In this study, out of 57 patients with parasitic infection 34(60%) were have cryptosporidia infection.Out of these 34 cryptosporidium positives cases, 26 (76 %) were male and 8 (24 %) were female.Among these, 25(73%) had <200cell/µlCD4 count and 9(27%) were with >200 cell/µl.

Table 1: Distribution of various types of intestinal parasitic infection

Parasites	No. of patients	Percentage
Cryptosporidia	34	60%
Microsporidia	9	15%
Cyclospora	4	7%
Isospora belli	3	5%
Entamoebahistolytica	3	5%
Ascarislumbricoides	2	4%
Entamoeba coli	2	4%
Total	57	100%

Table 1 shows, Out of total 70 HIV patients, 57 (81%) were positive for parasitic infection, higher number of parasitic infection is caused by Cryptosporidia in 34(60%) followed by Microsporidia 9(15%), Cyclospora 4(7%), Isospora belli 3(5%), Entamoebahistolytica 3(5%), Ascarislumbricoids 2(4%) and Entamoeba coli 2 (4%).

Table 2: Staining methods for identification of cryptosporidium(n = 34)

Staining method	No. of positive	Percentage (%)
Modified ZN stain	33	97
Safranine methylene blue stain	31	91
Auramine phenol stain	34	100

Table 2 shows, out of 34 cryptosporidium positive samples, cryptosporidia were found positive in 33(97%) and 31(91%) respectively in modified Z N stain and safranine methylene blue stain. All positive sample were confirmed by auramine phenol stain.

Table 3: Cryptosporidia and its correlation with diarrhea.

Coccidia	With diarrhea	Without diarrhea	Total
Cryptosporidia	22(65%)	12(35%)	34

Table 3 shows, out of total 34 patients with Cryptosporidium, 22(65%) had diarrhea with <200cell/µl while 12(35%) patients had no complain of diarrhea with>200cell/µl.

Discussion

Cryptosporidium is one of the most important causes of diarrhea in immunocompromised individuals and children. The significance of cryptosporidiosis depends on number of immunocompromised persons such as AIDS patients or consumers of immunosuppressive drugs.[3]

The staining techniques compared in this study were modified ziehlneelsen (MZN), safranine and auramine phenol stains. All the three identified satisfactorily oocysts of cryptosporidium in stool of both positive test and negative control.

Of the 34(100%) patients who had cryptosporidiosis were positive for auramine phenol technique, 33(97%) of them were also positive with the modified ZN stain and 31(91.0%) were also positive with the safranine stain. A.A Joseph and G.O Popoola[4] study also reveales the similar result of 80% and 81% respectively. Study carried out by Quadros R.M[7] shows auramine phenol detected 25 (100%) positive samples, whereas the Ziehl-Neelsen method detected 22 (80%) positive samples, with nostatistically significant difference. Similar result is also observed in our study, which showsAuramine phenol stain detected 34 (100%) positive and modified ZN stain detected 33(97%) positive sample.

In Safranine methylene blue stain, oocysts appeared as a bright reddish orange colourand the sporozoites within the oocysts stain slightly darker. Yeast, bacteria, fungal spores and other fecal debris took the counter stain methylene blue. Thus, the method has got the advantage over other methods in differentiating oocysts from yeasts and moulds[5]. Though safranine stain is rapid for detection of cryptosporidium, it requiresexpertization to differentiate from cyclospra stain by it.

The widely used technique in this environment is the modified ziehlneelsen stain hence the need to compare the other two methods with it.The reagents used in the modified ziehlneelsen staining is however readily available, affordable and cheaper compared to the reagents used in the other staining techniques being reviewed[4].

Auramine has a greater affinity for the Cryptosporidiumoocyst wall than fuchsin, a red dye used in ZiehlNeelsen staining technique. Auramine-stained oocysts withstand discoloration for 5 minutes, but oocysts stained by the ZiehlNeelsen technique exhibit complete discoloration within the same time frame.Theauramine phenol staining procedure gave the highest diagnostic yield but modestly expensive[7]. The disadvantages of this technique included its complexity, which required fluorescent microscope, frequent quality control monitoring and technological expertise to view auramine stained slide. In addition, the staining fluid is potentially carcinogenic, requiring processing in a fume hood and special handling for disposal[8].

The submission of Annam et al[6] was in support of this finding for they found fluorescent microscopy to be more advantageous than the ziehlneelsen for detection of cryptosporidiumoocyst. They concluded that the fluorescent microscopy has the advantage of speed and ease of screening and reduces observer fatigue. Study carried out by Quadros R.Met al[7] to detect cryptosporidium oocysts by auramine and ziehlneelsen staining methods, found that auraminehas a greater affinity for the cryptosporidium oocyst wall than fuschin used in ziehlneelsen. They concluded that auramine had more advantages over the ziehlneelsen method by being quicker, to perform, and read and ideal for population-based studies.

Conclusion:

In conclusionAuraminePhenol, a simple fluorescent staining, is highly sensitive, specific and less time-consuming. Thus for the diagnosis of intestinal cryptosporidiosis we can rely on Auramine Phenol staining technique.The study is helpful in conducting preliminary survey for screening of cryptosporidiosis in immunocompromised patients.

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