FREQUENCY OF CRYPTOSPORIDIUM IN CHILDHOOD DIARRHOEA- IMPORTANCE OF MODIFIED ACID FAST TECHNIQUE

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Background: One of the important causes of childhood diarrhoea is cryptosporidium, a parasitic pathogen which is usually overlooked. This study was therefore designed to show the importance of modified acid fast stain in the diagnosis of cryptosporidium in childhood diarrhoea. **Methods:** Stool samples from 300 children with prolonged diarrhoea were examined by modified acid fast staining along with wet mounts in saline and iodine. **Results:** Out of 300 samples five (1.7%) were diagnosed as positive for cryptosporidium. **Conclusion:** Results indicate that Cryptosporidiosis exists as a cause of diarrhoeal illness in our society. It was also noted that modified acid fast staining of stool samples is an important, non-invasive and useful diagnostic technique in identification of cryptosporidium.

Key words: Cryptosporidium. Childhood diarrhea, Modified acid fast technique

INTRODUCTION

Cryptosporidium is an enteric pathogen known to parasitise a wide range of animals and has a propensity to affect human beings.[1]

Early human cases were reported in the United States in 1976 in an immunosuppressed adult and an immunologically competent child.[2,3] An important aspect of cryptosporidiosis is its higher prevalence in young children with diarrhea.[4,5]

Molecular studies have shown that Cryptosporidium parvum is the major species involved in human infections.[6] Most infections are acquired through direct contact with infected persons or through contaminated water or food.[7,8]

The infection is intracellular. The organism replicates and matures in enterocytes. The final product is the oocyst that passes out in the stools. The infection is highly transmissible.[7,9] Medical history or physical examination are not sufficient for the diagnosis as diarrhoea is only a symptom caused by various pathogens through several mechanisms. However, cryptosporidium has been found to be significantly more prevalent in persistent diarrhea.[5,10]

Specific diagnosis depends on laboratory evaluation. Examination of stool for the characteristic oocysts by modified acid fast staining still remains an important diagnostic test.[11] Diarrhoeal illness is a leading cause of morbidity and mortality in children. Although a number of enteric pathogens have been identified in recent years but many episodes of diarrhoea still remain undermined. Cryptosporidium has emerged as an important enteric pathogen[10].

This study was designed to see the role of modified acid fast stain in the diagnosis of Cryptosporidium which is an unusual pathogen in children with prolonged diarrhoea.

MATERIAL AND METHODS

This study involved 300 children presenting with chronic diarrhoea who came during the course of this study. Inclusion criteria was stool samples from children aged 1-14 years presenting with chronic diarrhoea in cross sectional manner. Cases presenting with acute diarrhoea or suggestive of bacterial or viral infection were excluded. None of the patients were receiving any immunosuppressive therapy or steroid treatment.

Samples were collected in clean, dry, leak-proof glass containers. Each sample was examined for gross appearance. Presence of worm segments, mucus or blood was noted.

For microscopic examination wet preparations in saline and iodine were prepared and examined for ova or cysts.

Smears for modified acid fast staining were prepared by taking a pea sized material from the stool sample by a glass rod and making an even, thin smear on clean glass slide. It was left to dry and fixed with 70% alcohol.

After fixation smear was flooded with cold carbol fuchsin and left for ten minutes. Smear was decolourized with 1% HCl alcohol until colour ceased to flow. Smear was rinsed with water and counter stained with methylene blue for 30 seconds. The preparation was rinsed again with plain water, dried and examined under oil immersion objective. Oocysts of Cryptosporidium appear as bright rose pink spherules against light blue background. The modified acid fast technique is a sensitive diagnostic method as recommended by WHO.[11]

RESULTS

Out of 300 cases five were found to be positive for Cryptosporidiosis as shown in the table. History of clinical illness of all cases revealed prolonged, persistent diarrhoea. Five cases that were positive for Cryptosporidium did not give any history of nausea, vomiting or loss of appetite associated with diarrhoea. Stools were frequent and watery.

On gross examination of the five positive cases presence of worm segments or blood was not observed. In two cases mucus was present. In the remaining cases presence of blood and mucus was frequent.

Examination of stool samples by wet mounts failed to reveal oocysts of Cryptosporidium which were found positive on modified acid fast staining in five cases. Only one case positive for Cryptosporidium was also positive for Giardia cyst showing mixed infection.

Table-1: Clinical and Laboratory Findings in Cases of Cryptosporidiosis

Case No.	Age & sex	**Duration of** Diarrhoea	**Lab. Finding**		
			Gross	**Wet preparation**	**Modified acid fast stain**
1	7 yrs male	5 months	Loose watery	Giardia cysts +ve	Oocysts +ve
2.	8 yrs male	4 months	Loose water mucus +ve	No cysts or ova observed	- do -
3.	11 yrs male	3 months `	Loose	No cysts or ova	-do -
4.	8 yrs male	2 weeks	Loose	- do -	-do-
5.	10 yrs male	>1 week	Loose with mucus	-do-	-do-

DISCUSSION

The results of this study highlight the fact that unusual pathogens like Cryptosporidium should not be overlooked while treating cases with diarrhoea. Although the frequency was found to be 1.7% but it shows the existence of this unusual pathogen in our population.

Cryptosporidium has emerged as an important cause of diarrhoea world wide. This parasite has been reported not only from Western countries but also from Nigeria, Sudan, Srilanka, Bangladesh and India with a strong possibility for it to be present in Pakistan also.[10]

Cryptosporidium is recognized as a major cause of waterborne diarrhoeal illness and as a pathogen with long term effects on childhood growth and development.[12] The most common manifestation of this parasite was prolonged diarrhoea[13] as also seen in the aforesaid study.

In a study carried out on 63 Pakistani travellers to UK, oocysts of Cryptosporidium were found in 13 travellers presenting with diarrhea.[14] Another study was carried out on water samples in Lahore. Out of 70 water samples 9% were found to be positive for oocysts of Cryptosporidium.[15] In a study carried out on duodenal biopsies in Karachi, four cases were reported to be positive for Cryptosporidium. These cases presented with chronic diarrhoea and stool examination by routine wet mounts did not reveal any significant finding.[16]

Cases of prolonged diarrhoea are usually not referred by clinicians for laboratory diagnosis of Cryptosporidosis as most of the clinicians especially in Pakistan are still unaware of Cryptosporidiosis in immunocompetent children and they are not sure of the symptoms caused by it.

Cryptosporidium is an important etiological agent of childhood diarrhoea and should be identified in routine parasitologic tests of diarrhoeal stool samples.[17] Recognition and diagnosis of this pathogen is of utmost importance for the prompt treatment of such cases. Modified acid fast staining is a sensitive method that is economical and easily applicable as compared to invasive procedures like duodenal biopsies. This method can improve detection of common source out breaks as well as in constructing timely public health interventions.

CONCLUSION

Cases of cryptosporidium in childhood diarrhoea were diagnosed by modified acid fast staining technique. These positive cases were missed on wet mount. Modified acid fast staining was easily applicable and economical mode of diagnosis. Cases with persistent diarrhoea should be referred for diagnosis of Cryptosporidium

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