

Comparative Evaluation of Microscopy & Antigen Detection for Cryptosporidium by Elisa in Patients with Immunocompromised Status.

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Abstract:

Background: *Cryptosporidium* is a protozoan parasite with worldwide distribution mainly affecting children and HIV infected individuals with low CD4 counts. The infection is self limiting in immunocompetent hosts but can be severe and persist in the immunocompromised and malnourished individuals. The aim of this study was to estimate the prevalence of *Cryptosporidium* infection in faeces among immunocompromised patients

Methods

Faecal samples from 113 immunocompromised patients with diarrhoea and without diarrhoea were examined for oocysts by microscopy and *Cryptosporidium* antigen test was performed using ELISA method.

Results

Of the 113 stool samples our study indicated, 20.3% *Cryptosporidium* infection prevalence in the immunocompromised participated population. Furthermore, Modified ZN staining (Kinyon's) detected 13(11.5%), wet mount by direct microscopy 2(1.7%) and concentration method 4(3.53%) stool samples positive for oocysts of *Cryptosporidium parvum* and *Cryptococcal* antigen by ELISA 23(20.3%) Stool samples were positive.

Conclusion

The present study confirmed the high prevalence of *Cryptosporidium* antigen in fecal samples of immunocompromised patients in the region. As no chemotherapeutic agents have yet proven, especially in immunosuppressed patients, therefore our results highlight the importance of preventive intervention in these groups.

Keywords: *Cryptosporidium*, Oocysts, Kinyon's staining Coproantigen, Immunocompromised patients, ELISA

I. Introduction:

Cryptosporidiosis is caused by *Cryptosporidium* strains, a parasite classified as emerging pathogens. The organism infects the gastrointestinal epithelium to produce a diarrhea that is self-limited in immunocompetent persons, but potentially life threatening in immunocompromised persons (1). Furthermore, *Cryptosporidium* stated an organism with low infective dose and resistant to conventional water disinfectants (2). In addition, no definitive treatment and proven protocol has been achieved for cryptosporidiosis (3). Therefore, prevention seems to be the main effective disease protection, especially among immuno-compromised individuals.

Parasitic infections with *Isospora belli*, *Entamoeba histolytica* and *Cryptosporidium parvum* have been reported as being the most frequently identified organisms in India[4]. *Cryptosporidium parvum* is a small parasite measuring about 3-5µm in diameter. The life cycle of *Cryptosporidium parvum* is completed within the small intestine and colon of the host with the developing stages associated with the luminal surface of the intestinal epithelial cells where it remains intracellular but extracytoplasmic[3]. Transmission occurs by ingestion of contaminated food or water or contact with infected animals (beef, cattle, sheep, pig and dog). (5)

Cryptosporidium species are tiny microscopic parasites that cause cryptosporidiosis. *Cryptosporidium parvum* ingested as an oocyst, undergoes excystation and sporozoites parasitizes the host. The oocysts are highly resistant to common household disinfectants and survive for long periods in the environment [3]. Cryptosporidiosis is a major cause of diarrhoea in children with or without HIV infection in developing countries. AIDS and protein energy malnutrition are severely impairing the immune system, in such patients cellular immunity is low and implies that cellular immunity is the major component in eliminating the infection [6]. This is evident by correlation between lower CD4 T cells count, risk and severity of cryptosporidiosis [7]. It has major public health implication because infection can result from exposure to low doses of oocysts [7]. Identification of oocysts in stool samples have been described, these include modified Ziehl-Neelsen (ZN)

staining (2% sulphuric acid must be used) [8,9] Giemsa (GS) staining [10] and Sheather sugar flotation (SSF) technique [10-12]. The purpose of this study was to find out frequency of cryptosporidiosis, creating awareness among the population and to highlight the importance of detection of oocysts in routine stool examination and detection of Cryptococcal antigen by ELISA.

II. Material and Methods

A retrospective study was carried out in the Department of Microbiology for a period of one year, Overall, 113 immunocompromised patients including 9 with haematological malignancy, 32 renal transplant patients and 64 confirmed HIV⁺ patients were also participated. The patients were interviewed and a questionnaire was completed for demographical, hygienic, and environmental life style with special emphasis on the gastrointestinal symptoms as diarrhoea with duration, acute (<2 weeks) and chronic (>4 weeks) and persistent (2-4 weeks). Duration of immunosuppressive and antiretroviral therapy, relevant other laboratory investigations and specific laboratory test, such as HIV- ELISA by 3 kits, CD-4 counts were studied.

The patients were provided a wide mouthed, clean, dry, properly labelled plastic container for collection of stool samples. Five grams of formed or about 10ml of unformed stool was collected and samples were examined within one to two hours of collection. Wet mount by both direct and concentration method was done from stool samples and smears were prepared stained by modified Ziehl-Neelsen staining method. [8,9] The stained smears were examined under oil for oocysts of *Cryptosporidium parvum*.

A total 113 stool samples were collected and stored at -20°C without any preservative until used. The ELISA was used for *Cryptosporidium* antigen detection in stool samples. The samples were defrosted and enzyme immunoassay was performed as described by the manufacturer. The results were carried out spectrophotometrically through ELISA reader according to manufacturer guidelines.

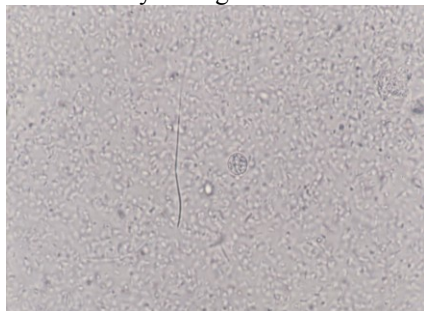


Figure 1: shows *Cryptosporidium* Oocyst Normal Saline mount (40X)

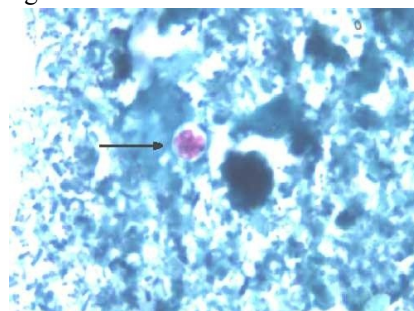


Figure 2: shows *Cryptosporidium* Oocyst, Modified Kinyon's Stain (100 X)



figure 3: *Cryptosporidium* ELISA Kit

III. RESULT

Among 113 immunocompromised patients, were included in the study mean age of the patients was 39.5 years though the range varied from 3-76 years.77% of the patients were between 21-50 years of age. A predominance of males(76%). Over females (24%) was observed male female ratio was 3:1.

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Among them 56.6% were from immunology ward followed by Nephrology(28%), Haematology (8%) and others (7%).[Table- 1]

Oocysts of Cryptosporidium were detected on direct wet mount 2, in 4 after Concentration Method and on applying Kinyon’s Staining 13 additional oocysts of Cryptosporidium were detected[Table-2].All Oocyst of Cryptosporidium were detected from patients with diarrhoea. Mixed infection with Oocyst of Cryptosporidium was found in 2 patients each with Larvae of Strongyloides *stercolaris* and Isospora respectively.

Cryptosporidium antigen detection in stool was detected by ELISA in 23/113(21%) as compared to microscopy in 13/113(11.5%)[Table 3]. On subgroup analysis, it was found that out of 64 HIV positive patients, 7/8 positive by microscopy were also positive by ELISA. 9/56 patients negative by microscopy was positive by ELISA. All patients with Cryptosporidium has CD4 count less than 200 cell/μl[Table 4]. Out of 32 Renal transplant recipient 4/5 positive by microscopy were also positive by ELISA and 3/27 patients negative by microscopy were positive by ELISA[Table 5].Among 9 patients with Hematological malignancies and 8 others no opportunistic were detected from any case.

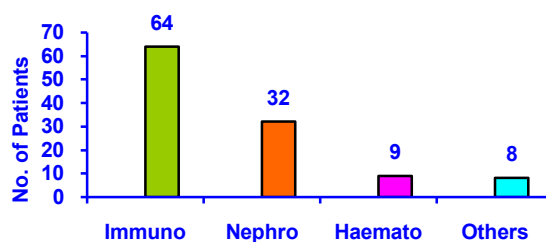


Table 1: Ward wise distribution of Immuno-compromised patients

Microscopy	(N=113) Direct		(N=113) Concentration method		
	Normal Saline	Iodine	Normal saline	Iodine	Kinyoun’s
Positive	2	2	4	4	13
	Cryptosporidium	Cryptosporidium	Cryptosporidium	Cryptosporidium	Cryptosporidium
Negative	111	111	109	109	100

Table-2 : Comparison of direct and concentration method.

Antigen	Microscopy		Total
	Positive	Negative	
Positive	11 (a)	12 (b)	23
Negative	2 (c)	88 (d)	90
Total	13	100	113

Table -3: Comparison of Microscopy and Cryptosporidium antigen detection in stool.

Antigen	Microscopy		Total
	Positive	Negative	
Positive	7	9	16
Negative	1	47	48
Total	8	56	64

Table 4 : Comparative evaluation of microscopy with antigen detection in HIV

Antigen	Microscopy		Total
	Positive	Negative	
Positive	4	3	7
Negative	1	24	25
Total	5	27	32

Table 5 : Comparative evaluation of microscopy and antigen detection for Cryptosporidium in renal transplant recipients

IV. DISCUSSION

Cryptosporidiosis is recognized as an important gastroenteritis disorder of immunocompromised patients. In such cases, it may lead to high morbidity and mortality.[12] The present work was designed to study the spectrum of Cryptosporidium in immunocompromised patients at our centre. A total of 113 patients were included in the study. Of these 86 were males and 27 were females. The male female ratio was 3:1. Similar finding of male preponderance has been quoted by many workers.[14] They found a male and female ratio of 3:1 when detected Cryptosporidium infection in 22 patients. 72.7% were male and 27.3% were female, which was almost similar to our study. Similar result was also found by Mohandas *et al.*[13] However, few have reported 5:1 male: female ratio, when they detected Cryptosporidium infection in 60 renal transplant patients.[15,16]

In this study, On direct wet mount preparation only were 1/23 Oocysts of Cryptosporidium could be detected as compared to that after concentration 2/23. On applying special staining method (Kinoyun's modified acid fast stain), 9 Cryptosporidium and this difference was significant. A similar finding have been reported by Wiwanitkit V *et al* and Ziring D *et al* were found 50%, 48% of the total subjects infected with intestinal protozoa respectively.[17,18] Nomir *et al* reported that after concentration there was increase from 27% to 56% by concentration method. Similarly on applying special staining methods for the identification of oppurtunistic parasitic pathogen diagnostic yield increased to 55.5%.[19]

In the present study mixed infection was detected in 2/113 cases. One with Cryptosporidium and larva of Strongyloid and another one with Cryptosporidium and Isospora. This is important because even if we get one organism in sample it must be thoroughly screened, otherwise patient will not be completely cured.

Cryptosporidium antigen in stool was detected by ELISA in 23/113 (21%) as compared to microscopy in 13/113 (10.6%) and the difference was statistically significant ($p < 0.025$). Sensitivity, specificity, PPV and NPV of antigen detection was 84.62%, 88%, 47.83%, 97.7% respectively. Two samples were microscopically positive but negative antigen detection. There could be several reasons for the discrepancy in results between the different techniques detecting oocysts in feces. In faecal samples, it is probably also an advantage to test for a soluble antigen that may be present more uniformly in the feces than to detect oocysts which are intermittently excreted. Monoclonal antibodies produced against *C. parvum* are generally considered to be genus specific rather than species specific and till date there has been no reports on monoclonal antibodies that can be used to differentiate between oocysts of different species.[19,20] In rest nine cases microscopy and antigen detection both were positive. Antigen detection picked by additional 12 cases which were microscopically negative. This may be due to the fact the sensitivity of antigen detection is very high 30 ng/ml as compared to microscopy. Our results are in agreement with the finding of Bialek Ralf *et al* who reported sensitivity of ELISA of Cryptosporidium was 94% in his study and found antigen detection superior to microscopy.[21] But this is not in agreement with the finding of Nomir *et al* who did not find it to be much superior to microscopy.[18]

The present study confirmed the high prevalence of *Cryptosporidium* antigen in fecal samples of immunocompromised patients in the region. As no chemotherapeutic agents have yet proven, especially in immunosuppressed patients, therefore our results highlight the importance of preventive intervention in these groups.

ELISA showed high sensitivity and specificity with reliable results for *Cryptosporidium* antigen detection in stool [23]. In addition cryptosporial antigens may be present in stool without existence the whole oocysts [24], therefore its application in routine diagnosis and even large-scale epidemiological surveys is recommended.

V. CONCLUSION

On the bases of the observation in the present study, Most of the patients infected with HIV and those who undergo renal transplantation are young. Male are at higher risk. Opportunistic parasitic pathogens are common in immunocompromised patients. Opportunistic parasitic pathogen are often missed on routine stool examination. Strong clinical suspicion and special staining methods must be used for their diagnosis. Cryptosporidium is most common opportunistic pathogen. Cryptosporidium commonly presents as chronic diarrhoea. Most of these parasites are detected when the CD4 count is low (200 cell/ μ l). So immunocompromised patient with low CD4 count must be screened for oppurtunistic parasitic pathogen. Antigen detection in stool is a potentially useful method for the diagnosis of Cryptosporidium.

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