ORIGINAL ARTICE

Clinico-Epidemiological Profile and Utility of Diagnostic Techniques in Immunocompetent Children with *Cryptosporidium* Diarrhoea

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ABSTRACT

Purpose: To assess the Clinical and Epidemiological profile of children 6 months to 5 years having *Cryptosporidium* diarrhoea and to compare the diagnostic accuracy of rapid diagnostic test (RDT) against modified acid-fast stain technique to detect *Cryptosporidium* in stool. **Materials and Methods:** Details of demography, presenting complaints, and detailed examination of children presenting with acute or persistent diarrhoea were recorded in a predesigned proforma. Two Stool samples were collected and examined same day by rapid antigen test and microscopy for presence of antigen or oocyst of *cryptosporidium*. **Results:** Out of the total of 280 samples tested, 40 (14.3%) samples were positive for *Cryptosporidium*. The prevalence of *Cryptosporidium* in children presenting with diarrhoea in our study came out to be 14.3%. Fever, vomiting and pain abdomen were the most common presenting complaints. Epidemiological factors like providing clean water, proper feeding and hygiene, and avoidance of overcrowding can help in reducing the burden of Cryptosporidium diarrhoea. Persistent diarrhoea was more common among *cryptosporidium* positive cases. Rapid detection kits enhance the detection rate and are sensitive as well as specific. It could be utilised as an aid in diagnosis of *Cryptosporidium* diarrhoea. **Conclusion:** The study shows that *cryptosporidial* diarrhoea is not uncommon in immunocompetent children. Improvement in personal hygiene, feeding practices and access to safe and potable water can help in reducing burden of this disease. Antigen detection kits aid in early diagnosis.

KEY WORDS: Paediatric cryptosporidiosis, Persistent diarrhoea, Sanitation, RDT in diarrhoea, Abdominal pain.

Introduction

Diarrhoea is the third leading cause of mortality among children under five years of age.[1] *Cryptosporidium spp*. is second only to rotavirus as the most common cause for moderate to severe diarrhoea in children under five years of age, as reported in Global Enteric Multicentre Study (GEMS), 2013.[2] It affects both immune-competent and immunocompromised individuals, with chronic course of illness seen in the latter. Despite being a major cause of waterborne outbreaks worldwide, Cryptosporidiosis remains a less described clinical entity and thus was included in the WHO Neglected Disease.

However, the prevalence varies in different parts of the country. In South India, *Cryptosporidium spp*. was detected in 13.1% of children with diarrhea and 9.1% of age matched controls.[3]Reports from West Bengal and Mumbai showed the parasite to be prevalent in 4.45% and 5.5% of children with diarrhoea respectively.[4,5] Prevalence rate of 1.3%-27% was reported from North India[6,7]. *Cryptosporidium* infection can present as symptomatic or asymptomatic childhood infection.

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Children with *Cryptosporidium* diarrhoea usually present with fever, nausea, pain in abdomen and abdominal distension however dehydration is uncommon.[8] Infection is self-limited in immunocompromised patients, but persistence of infection may lead to growth faltering in children. Environmental factors, such as sanitation, hygiene, source of drinking water, climatic condition, household etc. also play a role in the transmission and propagation of this disease and have a dominant role in its epidemiology. Malnutrition also poses a risk for higher susceptibility to *Cryptosporidium* infection.[9]

Despite having a high burden of morbidity and mortality, detection and isolation of *Cryptosporidium* is a challenging task. It is done by examination of stool samples which is a cumbersome process and sometimes requires examination of more than one sample over several days. Owing to limited studies in our part of country, the present study was proposed to study the clinical and epidemiological profile of immunocompetent children with *Cryptosporidium* diarrhoea and to study the performance of rapid detection test (RDT) against conventional microscopy using acid-fast staining techniques.

Methods

This is a prospective study, carried out in a tertiary care teaching hospital, in children aged 6 months to 5 years, admitted with acute or persistent diarrhoea. The study period was from December 2018 to February 2020. The Institutional Ethical committee approved the study and written informed consent from guardians of children was taken. The calculated sample size was 280 children, based on our assumption of prevalence of *Cryptosporidium parvum* as 27.4% from a study in North India by Bera et al.[7]

Children who already received antibiotics or anti parasitic drugs before coming to hospital, with history suggestive of immunocompromised status viz., recurrent skin and respiratory infections, or any known infection with unusual fungal or parasitic organisms or with history of steroid intake in past three months were excluded.

The details of demography, epidemiology and the presenting complaints along the examination including anthropometry were recorded in a pre-designed proforma. Two stool samples were collected in universal containers and examined on the same day. One by using commercially available kit (CoproStrip^{TM} *cryptosporidium* by Savyon diagnostics) which is a rapid chromatographic immunoassay for qualitative detection of *cryptosporidium* antigen and the other by microscopy using Modified Zeihl Nelson technique for staining to detect the presence of *Cryptosporidium* oocyst.

Data was analysed using Statistical Package for Social Sciences (SPSS) version 21.0. Data was represented as frequencies (numbers) and proportions (percentages). Central tendency was depicted as mean ± standard deviation. Chi-square test was used to compare the data. A 'p' value less than 0.05 was considered as significant.

Result

Out of 280 patients enrolled in the study, a total of 40 were positive for *Cryptosporidium* (CP) while remaining 240 (85.7%) were negative. The prevalence of *Cryptosporidium* diarrhoea came out to be 14.3% in our study. Among positive cases 55% were males. Higher positivity rate was seen in age group 12 to 24 months (47.5%) and in patients from rural areas (55%). Clinical features of CP positive cases are shown in the Table 1. Fever, vomiting, and pain abdomen were the most common presenting complaints. Among positive cases 47.5% were moderately underweight (Weight for age below – 2SD) and 37.5% were stunted (height for age less than -2SD). Maximum number of cases presented between March and June (37.5%) and majority presented with acute diarrhoea. (70:30 acute: persistent).

Epidemiological factors are listed in Table 2. Among all factors overcrowding and bottle feeding was found to be statistically significant in contributing towards *Cryptosporidium* infection. Most of the positive cases belonged to upper lower class (Kuppuswamy class IV) thus reflecting the impact of poverty, overcrowding, poor sanitation, and limited access to potable water in these families. Comparison among CP and CN patients showed that 30% of CP patients had persistent diarrhoea whereas only 9.2% CN had persistent diarrhoea which was statistically significant ($p<0.001$).

Stool sample was tested with antigen detection kit and microscopically. *Cryptosporidium* was detected in 40/280 cases by RDT. Microscopy alone was not successful in detecting any case. Both microscopy and RDT was positive in 26 out of 40 cases. Thus,

Table 1: Clinical profile of children

SN	Characteristic	*Cryptosporidium* positive (n=40)		*Cryptosporidium* negative (n=240)		Total (n=280)		Statistical significance	
		No.	%	No.	%	No.	%	χ^2	'p'
1.	Fever	30	75.0	162	67.5	182	68.6	0.895	0.344
2.	Vomiting	28	70.0	157	65.4	185	66.1	0.321	0.571
3.	Pain abdomen	30	75.0	64	26.7	94	33.6	35.916	<0.001
4.	Abdominal distension	30	75.0	49	20.4	79	28.2	50.434	<0.001
5.	Blood in stools	0	0	24	10.0	24	8.6	4.375	0.036
6.	Reduced Appetite	1	2.5	9	3.8	10	3.6	0.156	0.693
7.	Acute diarrhea (< 14days)	28	70	218	90.8	246	87.9	13.9 p<0.001	
8.	Persistent diarrhea (>14days)	12	30	22	9.2	34	12.1		
9.	Pica	5	12.5	33	13.8	38	13.6	0.046	0.831
10.	**Dehydration**								
	No	3	7.5	12	5.0	15	5.4		
	Some	37	92.5	223	92.9	260	92.9	1.234 p=0.540	
	Severe	0	0.0	5	2.1	5	1.8		
11.	**Modified Vesikari Score**								
	Mild	3	7.5	12	5.0	15	5.4		
	Moderate	37	92.5	223	92.9	260	92.9	1.234 p=0.540	
	Severe	0	0.0	5	2.1	5	1.8		

emphasizing that as compared to microscopy RDT was 100% sensitive, 89.6% specificity, had a 65% positive predictive value, 100% negative predictive value and had a 95% diagnostic accuracy. Moreover, results are obtained rapidly and do not require additional reagents and instrumentation.

Discussion

Diarrhea is the third most common cause of death in under-five children, responsible for 13% deaths in this age-group, killing an estimated 300,000 children in India each year.[10] Despite being a major cause of waterborne outbreaks worldwide, Cryptosporidiosis remains a less described clinical entity and thus was included in the WHO Neglected Disease Initiative in 2004 along with other diseases which have a common link with poverty and nutritional compromise[10]. The prevalence of cryptosporidium among suspected children is varied, ranging as low as 2.7%[11] to as high as 27.4% depending on the method or technique used to diagnose the cases.[5] Prevalence of *cryptosporidium* was 14.3% in this study.

The majority of *Cryptosporidium* diarrhea cases were <24 months of age in this study (67.5%), which is similar to other studies where maximum burden is reported in children <2years.[12] Seemingly the diminishing levels of maternal antibody and increased exposure to pathogens by virtue of feeding practices in this age make them more susceptible to infections. Gender dominance is variable[12] as reported in different studies showing both male and female preponderance. In this study both males and females were almost equally affected. The gender-related difference in prevalence seems incidental.

In present study severity of illness was adjudged using Modified Vesikari score[13], according to which 5.4% had mild diarrhoea, only 1.6% had severe diarrhoea and majority i.e.92.9% had moderate diarrhoea.

Being a disease with feco-oral transmission, role of many epidemiological factors affecting living conditions and hygiene makes it a disease of public health importance. This study shows children of rural family background and those belonging to low socio-economic class are likely to carry this infection as compared to those from urban background. Although this result was not statistically significant it was consistent with findings from previous studies.[14] Considering the living condition and household and family size, the majority of positive cases lived in small house with inadequate ventilation and faced

Table 2: Epidemiological profile

SN	Characteristic	*Cryptosporidium* positive (n=40)		*Cryptosporidium* negative (n=240)		Total (n=280)		Statistical significance	
		No.	%	No.	%	No.	%	χ^2	'p'
1.	**Source of water supply**								
	Tap water	18	45.0	149	62.1	167	59.6	4.498	0.105
	Handpump	21	52.5	84	35.0	105	37.5		
	Tube well	1	2.5	7	2.9	8	2.9		
2.	**Water cleaning method**								
	Boiling	2	5.0	30	12.5	32	11.4	2.884	0.410
	Filter	0	0.0	5	2.1	5	1.8		
	Reverse osmosis	2	5.0	11	4.6	13	4.6		
	None	36	90.0	194	80.8	230	82.1		
3.	Open sewage disposal	23	57.5	112	46.7	135	48.2	1.612	0.204
4.	Open air defecation	22	55.0	95	39.7	117	41.9	3.2773	0.070
5.	**Waste disposal**								
	Near	18	45.0	145	60.7	163	58.4	3.464	0.063
	Away	22	55.0	94	39.3	116	41.6		
6.	**Handwashing**								
	Running water	18	45.0	143	59.8	161	57.7	3.109	0.211
	Bucket	21	52.5	91	38.1	112	40.1		
	Others	1	2.5	5	2.1	6	2.2		
11	**Housing**								
	Pucca	18	45.0	146	60.8	164	58.6	3.542	0.060
	Kutcha	22	55.0	94	39.2	116	41.4		
12	**No. of rooms**								
	1	13	32.5	58	24.2	71	25.4	2.392	0.302
	2	27	67.5	174	72.5	201	71.8		
	3	0	0.0	8	3.3	8	2.9		
13	Over crowding	40	100	16	6.7	56	20.0	186.67	<0.001
14	Inadequate ventilation	10	25.0	61	25.4	71	25.4	0.003	0.955

overcrowding (p=<0.001), which is again in accord to other studies.[15]

Previous studies have revealed limited potable water and poor sanitation facilities.as a risk factor for Cryptosporidiosis..[12] [15]In this study also in majority of *Cryptosporidium* positive cases the source of drinking water was from hand pump (52.5%) and 90% did not use any water cleaning measure before drinking. Open sewage disposal was present in 60% patients, 55% defecated in open, 52.5% used to wash hand in water stored in open bucket and 45% had waste disposal in the vicinity of house. Thus, highlighting the need to strengthen the public health measures. There is a need to improve and provide health education, safe water and sanitation facilities especially to the rural cohort.

Cryptosporidiosis has been associated both with acute and persistent diarrhea in the present study.[16,17]

As suggested by many studies, rainy season is most favourable for this parasitic infection[18]however, some studies have reported a higher prevalence in summers. [19] In this study also more cases presented in summers (37.5%) when compared to rainy and winter season. Higher case load in summers can be due to prolonged summers in eastern Uttar Pradesh along with poor sanitation, poor hygiene and low socioeconomic conditions.

In most recent studies, a significant proportion of healthy children with diarrhea were detected positive for *cryptosporidium*. The protozoan was also detected in mixed infections. Thus, screening for *cryptosporidium* should be contemplated in settings of prolonged or persistent diarrhea. Lack of specific clinical signs or pattern of illness also justifies its screening. In addition to diarrhea, *cryptosporidium* had significant impact on childhood growth in both symptomatic and asymptomatic infections with greater severity in symptomatic infection than asymptomatic infection.[20]

Feeding practices have been shown to affect the prevalence of Cryptosporidial infection in children.[21] This study found the practise of bottle feeding in infants to be an important risk factor. As found in this study *Cryptosporidium* infection was more common in malnourished children and it further leads to chronic malnutrition thus it creates a vicious cycle that results in growth failure and stunting,[11] even if the child is asymptomatic.[22]

Although microscopic examination is routinely used for diagnosis of *Cryptosporidium*, however, it requires high level of skill and infrastructure and is time-consuming, thus delaying the diagnosis and further the specific management of the patient. Rapid diagnostic tests (RDTs) relying on antigen detection from stool could provide immediate results without the need of highly skilled personnel. A multi-centre study evaluating[23]four commercial rapid immunochromatographic assays showed a lower sensitivity (range between 47.2% to 70.6%) but a high specificity (100%). In our study, use of RDT helped to increase the positivity rate. As compared to microscopy RDT was found to have 100% sensitivity, 89.6% specificity, 65% positive predictive value, 100% negative predictive value and 95% accuracy. The reasons for low microscopic yield in this study could be presence of less parasitic load children were immunocompetent and rampant pre-treatment with over the counter antibiotics.

This study provided a clinical and epidemiological insight of *Cryptosporidium* diarrhoea in our settings. The findings are unique from the point of view of high symptomatic load and high RDT sensitivity. Possible reason for this could be referral to tertiary care centre in a condition where they could not be managed at home or primary or secondary care centres. The findings thus indicate towards a possible higher load of *Cryptosporidium* diarrhoea in community than in hospital settings. Future studies directed in estimating the actual burden of *Cryptosporidium* diarrhoea in urban settings should be carried out as collaborative studies involving primary and secondary caregivers too.

Limitations

This study is limited to a hospital setting thus covering only a small population a community-based study is required to assess the actual prevalence of disease.

Conclusion

This study highlights the fact that *cryptosporidium* diarrhea is still a neglected disease. *Cryptosporidium* was the cause of diarrhea in 15% of immune competent children in this study. Younger children (<2 years) from lower socioeconomic strata exposed to poor sanitary and hygiene conditions is the vulnerable population. Acute diarrhea with pain in abdomen and distension are the differentiating clinical features from other causes of diarrhea. Use of rapid detection kits enhances the detection. Hence this study recommends creating awareness and understanding of this pathogen as a cause of childhood diarrhea among paediatricians. Thus hoping to improve and strengthen the overall diagnostic and therapeutic algorithm of childhood diarrheal illness

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