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PREVALENCE OF CRYPTOSPORIDIOSIS IN WESTERN INDIA

Medical Microbiology

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ABSTRACT

Introduction: Cryptosporidium hominis, Cryptosporidium parvum, Cryptosporidium meleagridis, Cryptosporidium felis, and Cryptosporidium canis are the five main Cryptosporidium species that cause infection. Among these five species C. hominis and C. parvum are most common which account for 95% of human cases of cryptosporidiosis. This study aim to estimate prevalence of Cryptosporidiosis in Western India. **Methodology:** The study is a cross-sectional study involving patients attending hospital with gastro-intestinal symptoms. Stool samples were received from patients admitted in various clinical departments as well as patients visiting OPD with complaints of diarrhoea for a period of 1 year. **Results:** A total of 100 stool samples were received out of which 67 were male and 33 were female patients. About 20 patients had presence of oocyst of cryptosporidium in their stool samples by iodine and saline mount examination followed by Modified Acid-fast staining & the highest number of cases was seen in the age group of 1-5 year (35%). The highest association between HIV seropositivity and cryptosporidium infection was seen among older patients (50%) followed by the adult age group of 18-40 years. The age group of 1-5 year showed 28.6% prevalence.The highest number of cases was seen among the lower class. ELISA was found positive in 25 patients for Cryptosporidium parvum antigen. And the highest number of positivity by ELISA was found in the age group of 1-5 years. **Conclusion:** Cryptosporidiosis is prevalent globally and has a significant impact on immunosuppressed individuals.

KEYWORDS

Cryptosporidium, Wet mount, ELISA

INTRODUCTION

Cryptosporidiosis is a diarrheal disease caused by the Cryptosporidium species, a protozoan parasite that infects both humans and animals.[1]Cryptosporidium was discovered by Tyzzer in 1907, but it was regarded as a non-pathogenic organism until 1976 when two human instances of cryptosporidiosis presenting with diarrhea were described and thus it was discovered to be an opportunistic pathogen.[2] In 1982, cryptosporidium was identified as a cause of self-limiting diarrhea in the general population with a healthy immune system and a cause of life-threatening disease in immunocompromised individuals, including those taking immunosuppressive drugs or suffering from Acquired Immunodeficiency Syndrome (AIDS).[2]

Cryptosporidium species is now commonly identified as a cause of acute, self-limited diarrheal illness in immunocompetent hosts in both developed and developing nations.[3]There are more than 120 genotypes and 44 species of Cryptosporidium identified so far.[4]Cryptosporidium hominis, Cryptosporidium parvum, Cryptosporidium meleagridis, Cryptosporidium felis, and Cryptosporidium canis are the five main Cryptosporidium species that cause infection. Among these five species C. hominis and C. parvum are most common which account for 95% of human cases of cryptosporidiosis.[4]

The most frequent reported risk factors of cryptosporidiosis are crowding, household diarrhea, poor quality of drinking water, wildlife exposure, open defecation etc. Cryptosporidium spp. has been ranked as the leading global cause (>50%) of waterborne outbreaks of protozoan infection.[4]

The feco-oral route is the primary mode of disease transmission. Oocysts containing four sporozoites are the infective form. The typical infective dose is 312 oocysts; however, as few as 10 oocysts might cause illness.

After the entry of cryptosporidium oocysts into the gastrointestinal system, the invasive Cryptosporidium sporozoites destroys the small intestinal epithelium, affecting intestinal barrier function and absorption capacity and producing mild to severe diarrhea and other gastrointestinal symptoms. In immunocompetent patients, cryptosporidium infection appears to be a self-limiting disease with no or moderate symptoms.[4] In addition to this transmission of Cryptosporidium can also occur via respiratory secretions or coughing. Lung infections have also been documented.[2,5]

The Center for Disease Control and Prevention (CDC) has classified cryptosporidium as a Category B bioterrorism agent because of its high infectious and transmissible ability.[6]Currently Nitazoxanide is the only FDA-approved drug for the treatment of Cryptosporidiosis, which has shown moderate efficacy in young patients but it is completely ineffective in immunocompromised people.[7]

MATERIALS AND METHODS:

The study is a cross-sectional study involving patients attending hospital with gastro-intestinal symptoms.Stool samples were received from patients admitted in various clinical departments as well as patients visiting OPD with complaints of diarrhoeafor a period of 1 year. Ethical clearance was taken.

The stool samples were collected and one part of the sample was transferred to a separate container to be stored at -20°C for the detection of Cryptosporidium parvum-specific antigen by ELISA. From another part of the sample both iodine and saline mounts were done on the stool samples to assess the presence of pus cells, RBCs, macrophages, protozoal cysts, helminthic ova and larvae.After wet mount all the samples were concentrated by Sheather sucrose floatation method. Smears were made from the concentrated stool and Modified acid fast stain (Kinyoun method) was done to assess for the presence of cryptosporidial oocyst in the samples.

The cryptosporidium oocyst was described as round to oval acid-fast oocyst, 4-5µm in size under oil-immersion field. In-vitro enzyme immunoassay for the qualitative detection of Cryptosporidium parvum-specific antigen was done by Serazym Cryptosporidium parvum developed by Seramun Diagnostics GmbH.

RESULTS:

A total of 100 stool samples were received out of which 67 were male and 33 were female patients. (Table No.1)

Table No.1: Gender-wise distribution of patients

Gender	Number of patients	Percentage (%)
Male	67	67
Female	33	33
Total	100	100

Out of the 100 stool samples tested 20 patients had presence of oocyst of cryptosporidium in their stool samples by iodine and saline mount examination followed by Modified Acid-fast staining. And among those 20 cases, the highest number of cases was seen in the age group of 1-5 year (35%) followed by 30% were from 18-40 year age group and 20% were from >40 year age group. (Table No.2)

Table No.2: Age-wise distribution of isolation of cryptosporidium oocysts in stool sample

Age group	Total no.of stool samples received from patients	Total no.of patients with oocyst of cryptosporidium	Percentage (%)
1 year	8	1	5%
1-5 year	14	7	35%
5-12 year	15	1	5%
12-18 year	4	1	5%
18-40 year	43	6	30%
>40 year	16	4	20%
TOTAL	100	20	100

Out of the 20 cryptosporidium positive cases 6 patients were found to be HIV positive by screening test.The highest association between HIV seropositivity and cryptosporidium infection was seen among older patients (50%) followed by the adult age group of 18-40 years. The age group of 1-5 year showed 28.6% prevalence. (Table No.3)

Table No.3: HIV seropositivity in patients with cryptosporidiosis

Age group	Total no.of patients with oocyst of cryptosporidium	HIV positive cases	Percentage (%)
1 year	1	0	0
1-5 year	7	2	28.6
5-12 year	1	0	0
12-18 year	1	0	0
18-40 year	6	2	33.33
>40 year	4	2	50
Total	**20**	**6**	**100**

To assess the risk factors associated with cryptosporidium in 14(70%) immunocompetent patients the patients were further evaluated for their socio-economic status according to Modified Kuppuswamy scale. And the patients were divided into three classes: Upper class, Middle class and lower class for the convenience of study. (Table No.4)

Table No.4: Modified Kuppuswamy classification of immunocompetent patients with cryptosporidiosis

Socioeconomic class	No.of patients	Percentage (%) N=14
Upper	2	14.29
Middle	5	35.71
Lower	7	50
Total	14	100

The highest number of cases was seen among the lower class (50%) followed by middle class (35.71%) and upper class(14.29%). Thus we can conclude that there is high impact of lower economic standards on the case prevalence as risk factors like overcrowding, improper sanitation and lack of filtered drinking water will be highest among them. (Table No.5)

All the 100 stool samples were subjected to Cryptosporidium parvum-specific antigen detection by ELISA. ELISA was found positive in 25 patients for Cryptosporidium parvum antigen. And the highest number of positivity by ELISA was found in the age group of 1-5 years.

Table No.5:Comparison of Cryptosporidium positive cases by ELISA and wet mount.

Age group	Total no. of stool samples received from patients	Total no. of Cryptosporidium positive patients by wet mount	Total no. of Cryptosporidium positive patients by ELISA
1 year	8	1	0
1-5 year	14	7	10
5-12 year	15	1	1
12-18 year	4	1	1
18-40 year	43	6	7
>40 year	16	4	6
TOTAL	100	20	25

DISCUSSION:

The prevalence of human cryptosporidiosis varies widely in different parts of the world.The disease is endemic in developing countries.[8,9] In India, the reported prevalence differs in various part of the country. In South India, Cryptosporidium spp. was detected in 13.1% of children with diarrhea.[10] Reports from West Bengal showed the prevalence of 4.45%.[11] Whereas in Mumbai the prevalence was found to be 5.5% of children with diarrhea.[12] North India showed a lower prevalence rate of 1.3%[13].

In the present study, Cryptosporidium was prevalent in 50% of adults 45% of children and 10% of infants. Whereas a study by Seema et al in Southern India showed the prevalence of Cryptosporidium in 35-36% of adults 17% of children and 20% of infants.[3]

Prevalence of cryptosporidiosis in HIV infected adults in this study was 20%. This correlates with a study conducted by Gupta et al in 2008 in New Delhi.[14] Other studies conducted by Mamatha et al.(Manipal)[15], Vajpayee et.al(New Delhi)[16], Shenoy et al.(Mangalore)[17], Banerjee et al(Vellore)[18] and Kumarasamy et al. (Chennai)[19] showed a slightly lower prevalence of 18.57%, 18.6%, 17.5%, 15.5%, 16% respectively. Whereas the studies conducted by Ghorpade et al(South Maharashtra)[20], Agarwal et al.(Imphal)[21] and Dwivedi et al.(New Delhi)[22] showed a considerably increased incidence of 83.3%, 81.8%, 66.6% respectively.

In this study the prevalence of cryptosporidiosis in children in the age group of less than 5 years was found to be 36.36% by wet mount & 45.45%by ELISA which is significantly higher than the studies conducted by Sengupta et al.(Kolkata)[23], Pherwani et al.(Mumbai)[24], Pal et al.(Kolkata)[25], and Nath et al.(Varanasi).[26] The prevalence reported in these studies were 7.1% , 4.4%, 6.8%, and 3.8% respectively.

The rationale for the significantly increased prevalence of this infection in children less than 5 years of age is due to their low immune functions and so a low dose of infection may easily result in Cryptosporidiosis.And in older children repeated low dose infections may induce immunity against Cryptosporidium spp.[27]

Maximum number of children infected with Cryptosporidium were from low socio-economic group which correlates with a study conducted by Nagamani et al.[28]

Detection rates of Cryptosporidium by wet mount examination, modified Ziehl Nelson and ELISA were found to be 55% and 75% and 100 % respectively[29,30].

CONCLUSION:

Cryptosporidiosis is prevalent globally and has a significant impact on immunosuppressed individuals. However, present control strategies, particularly diagnostic technologies, have significant limitations. Microscopy is the primary method for detecting cryptosporidia in feces. However, the diagnostic accuracy of these procedures is largely dependent on the microscopist's expertise. To avoid the spread of infections, rapid detection requires sensitive, specific, easy, cost-effective, and high-throughput molecular tools. Cryptosporidiosis can occur in both immunocompetent and immunocompromised individuals, but it is more severe in the latter.

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