

Cryptosporidium : A Cause for Hospital Admission Among HIV Patients at Federal Medical Centre (FMC), Keffi, Nasarawa State, Nigeria.

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Abstract:- The prevalence of *Cryptosporidium parvum* in people infected with HIV/AIDS at the Federal Medical Centre, Keffi was an infection in relation to sex and age of the immunocompromised patients. 200 stools were diagnosed and analyzed from patients infected with HIV within 1½ hrs after collection. The pathogenic *C. parvum* was diagnosed using a modified–acid–fast (Ziehl-Nelson) given that *C. parvum* has been known to be difficult to characterized using standard microbiological techniques. Here, 29 (14.5%) cases were positive with *C. parvum*, 14(7.0%) were found to contain mucous in the stool texture, 20 (10.0%) were however, diarrhoeic and 4 (2.0%) cases both contained mucous and diarrhoeic in texture. Also in the males 4 (11.1%) were positive with *C. parvum* oocysts, higher in the females 25 (15.3%), cases were found positively significant in relation to sexes ($X^2 = 0.577 < 0.05$). among the ages of 20 – 39 years as sexually active group were high with *C. parvum* oocysts with significant difference among the age groups ($X^2 = 0.768 < 0.05$). However, *C. parvum* remains a major clinical problem in patients living with HIV infection and suggests here that, routine stool examination will significantly; benefit immunocompromised patients by reducing high morbidity and mortality rate thereby improving the quality of life.

Keywords: Protozoa. Parasite. Pathogenic. *Cryptosporidium*. HIV.

I. INTRODUCTION

Pathogenic *Cryptosporidium* is an emerging pathogen which belongs to the protist Phylum – Apicomplexa (CDC, 2009). It affects the intestines of mammals and is typically an acute short-term infection with adverse effect of causing self diarrhoea in healthy individuals and children alike. Also recognized as a cause of persistent and a prolonged diarrhoea in persons with acquired immunodeficiency syndrome (AIDS). The main symptom of *Cryptosporidiosis* is self-limiting diarrhoea in people with intact immune systems. In immunocompromised individuals, such as AIDS patients, the symptoms are particularly severe and often fatal. The source of the most endemic *Cryptosporidiosis* cases is human – to – human faecal-oral transmission, but infection may also result from animal-to-person and water borne transmission. *Cryptosporidium* oocysts are highly infectious, requiring only 101 to 103 oocysts to cause human disease (50% infectious dose, 102). Though, the mechanism by which *Cryptosporidium* cause diarrhoea includes a combination of increased intestinal permeability, chloride secretion, and malabsorption, which are all thought to be caused by the host response to infection. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once infected, exist in the small intestine and result in an infection of intestinal epithelial tissue.

More recently, studies using polymerase chain reaction (PCR) tests, *Cryptosporidium* species have been diagnosed in 6% of American travellers to Mexico. Studies in Brazil documented an infected rate of 90% for children under the age of 5 years who were living in slums, meanwhile, serologic and stool studies have documented high rate of infection in Latin-America, Africa, the Middle-East, and South Asia though, in persons with AIDS, the rate of *cryptosporidiosis* is higher in developing countries, ranging from 12-48% with diarrhoea. Electron microscopic studies made from the 1970s have shown the intracellular, although extracytoplasmic localization of *Cryptosporidium* species, in the DNA studies of *Cryptosporidium*, suggest a relationship with the gregarines rather than the coccidian. The taxonomic position of this group has not yet been finally agreed upon (Carenno et al., 1999). And *Cryptosporidium parvum* was sequenced in 2004 and was found to be unusual among eukaryotes in that, the mitochondria seem not to contain DNA (Abrahamsen et al., 2004) while in a closely related species, *Cryptosporidium hominis* has its genome sequences available (Xuet et al., 2004).

However, *Cryptosporidium parvum* is to be determined in people living with HIV/AIDS in relation to sex and age at the Federal Medical Centre, Keffi, Nigeria.

II. MATERIALS AND METHODS

Study area

Diagnostic method

Two hundred (200) stool samples were collected from each patient infected with HIV in sterile bottles container within one and a half hours after collection in accordance with institutional ethical guidelines, and all the stool samples for parasitological investigation were processed in the laboratory.

Stool examination

The pathogenic cryptosporidium were diagnosed by examination of stool specimens as stained with modified acid-fast (Ziehl-Neelson) since the organism has been known to be difficult to characterize using standard microbiological techniques (CDC, 2009). Modified acid-fast staining technique is a simple and effective method; the oocysts stain bright red against a background of blue-green faecal debris and yeast. Here, concentrated sediment of fresh stool was used.

Procedure

A smear with 1 to 2 drops of specimen on the slide were prepared air dried on a slide warmer at 60°C until dry (The thickness of all the smears were checked). This was fixed with absolute methanol for 30 seconds and stained with kinyoun's carbol-fuchsin for one minute and was rinsed briefly with distilled water and drained. The slides were then destained with acid alcohol for 2 minutes, rinsed again with distilled water was allowed to get dry then, the slides were counterstained with malachite green for 2 minutes, and was rinsed briefly with distilled water and drained. At this point, the slides were then dried on a slide warmer at 60°C for about 5 minutes and mounted with a cover slip using desire mounting media. In all, 200 fields were examined using x40 or higher magnification (to confirm internal morphology, x100 oil immersion objective was used). The chi square (χ^2) was used to established statistical significance in the prevalence of cryptosporidium parvum infection in relation to age and sex. Chi-square (χ^2) test was carried out using SPSS statistical package.

III. RESULTS

Prevalence of Cryptosporidium

This is the first study to report the prevalence of *Cryptosporidium parvum* in HIV patients attending Federal Medical Centre, Keffi. Twenty-nine HIV patients were microbiologically linked to the incidence of cryptosporidiosis with evidence of diarrhea (Table 1). Oocysts counts were highest in stool and where contamination occurred, infection have been noted and it has conclusively demonstrated that this specie can be a human pathogen. In 15(7%) cases of the examined samples were found to contain mucus and 20(10%) of the examined 200 samples were diarrhoeic in texture while 4(2%) cases both contained mucus and diarrhoeic.

Age and Cryptosporidium

Unusually for cryptosporidiosis there were no affected children identified under the age of 9 years old probably that, no children born of HIV affected parents. Though, among the age of 10-19 years old 3(19.7%) cases of Oocysts of *Cryptosporidium parvum* were established. The greatest differences in prevalence of infection among and within the age group 20-29 year olds, 10(15.6%) occurred in relation to differences in exposure to faecal contamination in food and /or water as the case may be. The prevalence was slightly high between 30-39 years old 12(16.4%) and no oocysts isolated within the age group of 40- 50 years. There was no significant difference between the age groups defined ($\chi^2 = 0.768$, $P > 0.05$) (Table 2).

Sex and Cryptosporidium

This prevalence has revealed some differences in the mode of infection and females were over-represented, quite high in medical attendance (Table 3). Most were adult males and females HIV stool samples subjected to laboratory

findings and 4(11.1%) were significantly positive with oocysts of cryptosporidium and high in females 25(15.3%) with no significant differences between both sexes ($\chi^2=0.77$, $P>0.05$).

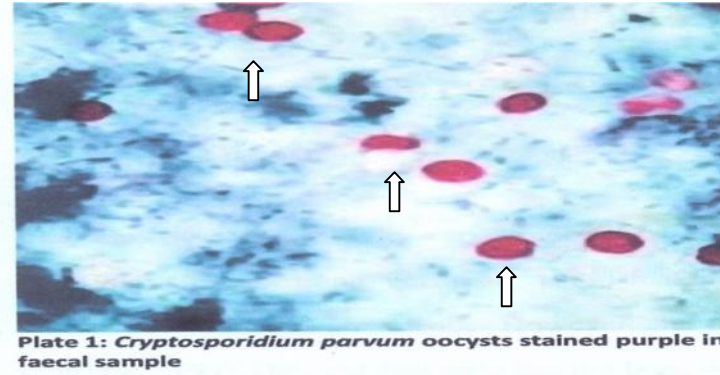


TABLE 1: PREVALENCE OF CRYPTOSPORIDIUM PARVUM AMONG IMMUNOCOMPROMISED PATIENTS WITH DIFFERENCE IN STOOL TEXTURE.

Total No. examined	No. positive	Mucous	Diarrhea	(+) mucous diarrhea	(-)mucous diarrhea
200	29	14	20	04	171
100%	14.5%	07%	10%	02%	85.5%

TABLE 2: AGE AND CRYPTOSPORIDIUM PARVUM INFECTION AMONG IMMUNOCOMPROMISED PATIENTS

Age-group	≤ 9	10-19	20-29	30-39	40-49	≥50 yrs	Total
No. of samples examined.	0	18	64	73	36	09	200
No. positive.	0	3	10	12	04	0	29
Percentage (%)	0.0%	16.7%	15.6%	16.4%	11.1%	0.0%	14.5%

(≤) =Less than or equal to. (≥) =Greater than or equal to. (%) =Percentage

TABLE 3: SEX AND CRYPTOSPORIDIUM PARVUM AMONG IMMUNOCOMPROMISED PATIENTS.

Sex	No. examined	No. positive	(%) percentage
Male	36	4	11.1%
Female	164	25	15.3%
Total	200	29	14.5%

IV. DISCUSSION

Given the potential for *Cryptosporidium parvum* to cause outbreaks of diarrhea in human populations, it was noted that, the rates of stool positivity varied, based on the acid-fast stain diagnostic techniques employed however, data indicate the ubiquity of the parasites as was noted, making it difficult to gain an accurate picture of incidence rates or of prevalence similar to the observation made by Bogaret *et al.*, (1984) and Current, (1994).

The pathogen *Cryptosporidium parvum* has contributed significantly to diarrheal disease particularly in countries of the world where a declining in hygiene can be a persistent problem, considering cryptosporidium an emerging protozoan and a leading cause for hospital admission at the federal medical centre (FMC), Keffi, Nigeria. The acid-fast was easily and cheaply carried out in a laboratory setting.

In this study, cryptosporidium are identified among patients affected with HIV reporting diarrheal disease, considered opportunistic with high risk tendencies. *Cryptosporidium parvum* infection was suggested to be low specific and sensitive (45.5%), compared to 33% global deaths caused by *Cryptosporidium parvum*, as a result diarrheal disease had occurred in up to 80% of persons with HIV (Bogaerts, 1984) which is agreed as a common phenomenon in some parts of Nigeria (Nwakediuko *et al.*, 2002). Moist and fluid environment becomes favourable for the growth of oocysts and this observation was most important to note the potential indirect effects of the HIV/AIDS epidemic which have implications to water, food and invasion potential. Casemore (1990) observed that, cryptosporidium is a major enteric pathogen of patients with AIDS, and with infection rates of 8-48% reported among African AIDS patients with diarrhea which 14.5% fall within the range of infection among HIV patients in Keffi.

Previous studies observed in patients with *C. parvum* to have experienced chronic diarrhea, while <15% (Manabe *et al.*, 1998, Ajjampure *et al.*, 2008) to have a transient diarrhoea or are asymptomatic, similar to observation made in this study where in any case patients with HIV developed chronic diarrhea disease, a cause for hospital admission. Though, the epidemiological surveys carried out in various parts of the world revealed that, the rate of cryptosporidiosis was anywhere from 3-5% (Bonilla *et al.*, 1992, Pedersen *et al.*, 1996). A number of studies indicated that the prevalence of cryptosporidiosis in HIV –infected patients was in the range of 15% or below (Florez *et al.*, 2003). Similarly, several studies have shown a high prevalence of cryptosporidian infection in children but in some quota, a study among 156 HIV infected Thai patients who had acute diarrheal illness showed that 12.8% had cryptosporidiosis (10.0% in males and 19.1% in females) (Saksirisampant *et al.*, 2002). However, in Nigeria a study carried out on 161 HIV-infected patients with chronic diarrhea revealed no cryptosporidium oocysts that, intestinal cryptosporidiosis is not common in HIV infected patients with chronic diarrhea in Enugu (Nwokediuko *et al.*, 2002).

In this study, females were more affected (15.3%), this may be explained by the fact that, large numbers of females attend the HIV clinic than males. This could not however agree with the study of hospitalized children in Delhi found with cryptosporidium predominantly in males (Mahajan *et al.*, 1992). It was observed that, females were prompt to those risk factors considered susceptible to infection similarly, UNICEF found that girls may be more susceptible to diarrheal disease as they are often feel less, given less nutrients food, provided with less health care and given more work (Doyle 1995).

Though, cryptosporidium infection which leads to diarrheal disease, a cause for hospital admission was observed as an Acquired Immunodeficiency Syndrome (AIDS), while infection in the immunocompetent person is normally self-limiting, co-infection with HIV/AIDS often results in chronic diarrhea, health *Cryptosporidium parvum* was observed to be a threat with in the ages 10-19 years old and in the sexually active age group of 30-39 years. Just as it

is in Botswana, diarrhea is a leading cause of morbidity and mortality, the majority of deaths occurring in this same age group of <5 years of age (pruss-ustun, 2007).

The difference between the results in this study and those of previous studies could be due to the absence of stool samples from the age group involve (9). The fact that *C. parvum* is more prevalent in children than in other age groups may account for the prevalence in the age 10-19 (CDC, 2009). *Cryptosporidium parvum* in immunocompromised patients, such as those with HIV or those undergoing immunosuppressive therapy, may not be self-limiting, leading to dehydration, emaciation and, in severe cases, death.

However, this pathogen has impacted females vulnerability to infectious disease generally and diarrheal in particular. Significantly, a number of important protozoan gastrointestinal tract pathogens are associated with diarrheal disease. Therefore, cryptosporidium, a protozoal pathogen of global importance is increasingly implicated as a leading course of diarrheal disease in developing nations, particularly Africa (Bogaretset *al.*, 1984).

As a necessity, routine examination of stool samples for *C. parvum* will significantly benefit immunocompromised individuals by reducing morbidity and mortality and hence, improving the quality of life. Though, the limitation of this study lacked accessibility to the initial data (CD₄ status) of each patients attending HIV clinic to enable us ascertain the present CD₄ status in compromised to WHO recommended and acceptable standard CD₄ status in patients with HIV/Cryptosporidium infection.

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