

Prevalence of *Cryptosporidium* Species in HIV Positive and Negative Patients Attending Hong General Hospital and Michika General Hospital, Adamawa State, Nigeria.

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ABSTRACT: The study was carried out to determine the Prevalence of *Cryptosporidium* species in HIV Positive and Negative Patients Attending Hong General Hospital and Michika General Hospital Adamawa State, Nigeria. A total of 647 stool and blood samples were collected, 346 from Hong and 301 from Michika from both HIV positive and negative patients. The stool samples were examined for Oocysts using modified Ziehl-Neelsen Acid Fast Stain and the blood samples for HIV confirmation test and CD4⁺ cells count. A Prevalence of 247(38.2%) was recorded with 135(20.8%) in HIV Positive and 112(17.3%) in Negative patients, significant at 5% probability level. Variation in gender and age shows a higher prevalence of 43.1% in females and 32.6% males which was significant at 5% probability level and between the age of 21-30(26.7%) in HIV positive patients and between the age of 1-20(40.1%), in HIV negative patients which shows a higher prevalence. Low CD4⁺ cells count was recorded in HIV positive patients with *Cryptosporidium* infection of 37(15.7%) which had CD4⁺ cells count <200 and 199(84.3%) had CD4⁺ cells count >200. In HIV Negative patients only 13(3.20%) had CD4⁺ cells <200 and 398(96.8%) had CD4⁺ >200. Therefore a reduction in CD4⁺ cells count is associated with immune suppression by HIV virus as well as *Cryptosporidium* infection and other immune deficiencies.

Keywords: *Cryptosporidium* species, HIV Patients, Michika, Hong, Nigeria.

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I. INTRODUCTION

Cryptosporidiosis is an enteric disease having a zoonotic nature caused by a coccidian parasite of the genus *Cryptosporidium* (Graczyk *et al.*, 1997). *Cryptosporidium* has been found infecting a variety of animals, such as cattle, sheep, rodents, cats, dogs, birds, fish and reptiles. Human infection occurs due to *Cryptosporidium parvum*. The species also infects domestic animals. In humans, infections without symptoms are common, especially in healthy or immunocompetent individuals. After an average incubation period of one week, diarrhoea develops that spontaneously resolves after two weeks. By contrast, patients with impaired immune system may develop profuse life threatening diarrhoea that is very difficult to treat with current available drugs. Transmission is mainly human to human or animal to human through contaminated water and food. *Cryptosporidium* oocysts can survive for months in moist soil or water and survive harsh environmental conditions (heat, cold and drought) for extended period of time. They can also survive disinfection procedures such as chlorination (European Centre for Disease prevention and Control, 2013). It has been estimated that in HIV/AIDS patients with diarrhoea, *Cryptosporidium parvum* infection ranges from 10-30% in developed countries and 30-50% in the developing countries (Peterson, 1993).

Cryptosporidium parvum has also been implicated in the etiology of extra-intestinal cryptosporidiosis causing cholecystitis, cholangitis and pancreatitis in HIV/AIDS infected patients. Generally, *Cryptosporidium parvum* is the most common opportunistic protozoan parasite that is at present well established and documented as a worldwide cause of diarrhoea in immunosuppressed individual or patients with several immune impairments such as in HIV/AIDS, *Diabetes mellitus*, acute leukaemia and other hematologic disorders, interferon deficiencies, graft transplants, hospitalised and malnourished individuals (Murray *et al.*, 2005). The severity of the disease is manifested in AIDS patients usually when the CD4⁺ cell count is below 200 cells/mm³

(McDonald, 2000). The number of CD4⁺ cells count is higher in HIV/AIDS patients with diarrhoea when intestinal Cryptosporidiosis is not involved. CD4⁺ cell counts are particularly low in patients with extra-intestinal Cryptosporidiosis, which may be as low as 55 cells/mm³ (Lopez-Velazquez *et al.*, 1995; Theodos, 1998). Flannigan *et al.*, (1992) reported that patients with CD4⁺ cell count of less than 180 cells/mm³ have persistent infection while patients with CD4⁺ cells count greater than 200 cells/mm³ have a transient or self-limited infection. However, some studies have indicated that self-limited Cryptosporidiosis might be associated with a more intact immune system as reflected by an absolute CD4⁺ cell count.

II. MATERIALS AND METHODS

Adamawa state is located in the North eastern part of Nigeria. It lies between latitudes 7° and 11° North of the equator and Longitude 11° and 14° East of the Greenwich Meridian. It is made up of 21 local government areas (Adebayo and Tukur, 1999). The study was carried out in Hong and Michika. They lie between latitudes 12° 55' to 13° 32' North of the Equator and 10° 02' to 10° 45' East of the Greenwich Meridian. The temperature ranges between 36-39 degree Celsius with a mean annual rainfall ranging from 900-1005mm. Most residents of Hong and Michika are predominantly farmers, traders and civil servants.

Rainfall in the area is mostly between the months April to November, just like any part of the country rainfall is controlled by the movement of tropical discontinuity (ITD). Generally, August and September constitute the wettest months and the incidence of flood and erosion are experienced, mostly during this period in the north east, planting activities begins from May (Adebayo, 2004).

The study was a hospital based study in which both HIV/AIDS positive and negative patients visiting Hong and Michika General Hospitals respectively were provided with the information on objectives and purpose of the study and its possible benefits to the society at large. Those who consented to the study were provided with sample bottles for stool specimen for investigation and blood samples were taken for HIV confirmation test and CD4⁺ cells determination.

A total of 647 stool and blood samples were randomly collected in clean specimen bottles from male and female patients attending Michika General Hospital and Hong General Hospital. Participants were directed on how to collect the fresh stool samples. A portion of the stool was preserved in 5% formalin. 2ml of venous blood were also collected in bottle containing EDTA.

Detection of *Cryptosporidium* oocysts in the concentrated stool was carried out using modified cold Ziehl Nelson staining technique. A concentrated smear of the stool was made on a clean grease-free slide and fixed in methanol for 3 minutes. The slide was immersed in cold carbolfuchsin and stained for 15 minutes; was thoroughly rinsed in tap water and decolorized in 1% HCL (V/V) in methanol for 10-15 minutes. After rinsing again in tap water, the slide was counter stained with 0.4% malachite green for 30 sec. The slide was air dried and observed under the light microscope using x40 objectives lens for the presences of *cryptosporidium* oocysts and confirmed with oil immersion objective as small pink to red spherules on pale green background.

Data obtained was analysed using SPSS 18.0 (correlation and chi-square) at 5% probability level for factors such as age, gender, local government residence, CD4⁺ cells count and prevalence of the parasite in the stool. Results were summarized using tables and simple percentages.

III. RESULTS

Prevalence of *Cryptosporidium* Species In Hong and Michika Areas

The prevalence of *Cryptosporidium* infection in Hong and Michika is shown in Table 1 below. It can be seen that of the total of 647 samples examined 247 (38.1%) were positive for *Cryptosporidium* and the infection is higher in Michika (21.9%) than Hong (16.2). The difference was statistically significant ($P > 0.05$)

TABLE 1: Prevalence of *Cryptosporidium* Species Among HIV Positive and HIV Negative Patients Attending Hong and Michika General Hospitals.

L.G.A	No. Tested	No. Infected	Infection Rate (%)
Hong	347	105	16.2
Michika	300	142	21.9
Total	647	247	38.1

$\chi^2 = 1.814$; $df = 1$; $P > 5\%$ Significant

Gender Based Prevalence of *Cryptosporidium* Infection in HIV/AIDS Positive And HIV/AIDS Negative Patients Attending Hong and Michika General Hospitals

Table 2 shows the prevalence of *Cryptosporidium* species in HIV/AIDS positive and HIV/AIDS negative subjects Attending Hong and Michika General Hospitals. Approximately more females (23.0%) than males (15.1%) were infected. The difference was however statistically significant ($P > 0.05$).

Table 2: Prevalence of *Cryptosporidium* Infection In HIV/AIDS Positive And HIV/AIDS Negative Patients in relation to sex (Gender).

Gender	No. Tested	No. Infected	Infection Rate (%)
Males	301	98	15.1
Females	346	149	23.0
Total	647	247	38.1

$\chi^2 = 10.53$; $df = 1$; $P > 5\%$. Significant

Table 3: Prevalence of *Cryptosporidium* infection Among HIV/AIDS positive and HIV/AIDS Negative patients in Hong and Michika in relation to Age and sex.

Age	HIV Positive (+ve)		HIV Negative (-ve)	
	Males %	Females %	Males %	Females %
1-10	11(4.45%)	16(6.47%)	23(9.31%)	21(8.50%)
11-20	10(4.05%)	18(7.28%)	13(5.26%)	16(6.47%)
21-30	13(5.26%)	23(9.31%)	6(2.42%)	11(4.45%)
31-40	8(3.23%)	15(6.07%)	4(1.69%)	7(2.83%)
41-50	7(2.83%)	7(2.83%)	2(0.81%)	5(2.02%)
51-60	3(1.21%)	3(1.21%)	1(0.41%)	2(0.81%)
61-70	0(0.00%)	1(0.40%)	0(0.00%)	1(0.41%)
Total	52 (21.0%)	83(33.6%)	49(19.8%)	63(25.5%)
	135(54.6%)		112(45.3%) = 247	

Correlation Coefficient r;

Age and male Positive Patients = 0.86
 Age and Female Positive Patients = 0.76
 Age and Male Negative Patients = 0.90
 Age and Female Negative Patients = 0.97

Significant at 5% Probability Level

IV. DISCUSSION

Immune-suppression as a result of HIV/AIDS infection and other immune impairments or deficiencies as well as poor hygienic practices were observed to be some of the factors responsible for *cryptosporidium* infection in the area. The relatively high prevalence of *Cryptosporidium* among HIV/AIDS positive than HIV/AIDS negative patients in the present study appear to be lower than that reported by earlier workers from South Africa 59% and 49% (Omoruyi, *et al.*, 2011) and that of (Erhabor, *et al.*, 2011), in Delta State Nigeria, they registered 57.0% and 62.0% respectively. However other investigators reported 27.9% in Apulia (Oguntibejuet *et al.*, 2006), which were similar with the present study. The observed difference may be as a result of the studies and reduction in CD4⁺ cell due to HIV virus, *Cryptosporidium* parasite other immune deficiencies and sample size which was larger in the present study than those presented by other workers. However both the males (15.1%) and females (23.0%) among HIV/AIDS positive and negative patients were significantly infected with *Cryptosporidium* species. HIV male patients (21.0%) and females (33.6%), HIV negative males (19.8%) and females (25.5%). This result indicates that HIV/AIDS positive patients were more exposed to the risk of infection with *Cryptosporidium* as compared to HIV negative patients and females recorded high prevalence of the infection in both HIV positive and negative subjects. This finding is contrary to Nel. *et al* (2010). This might be as a result of more females are being exposed to the infection as compared to males in the area of study.

The prevalence of *Cryptosporidium* species with relation to age and gender, revealed a high prevalence in female (9.31.0%) between the ages of 21-30 and males (5.26%), of the same age range in HIV positive patients while in HIV negative patients high prevalence was also recorded in females (8.50%) between the ages of 1-10 and males (5.2%) of the same age range, therefore it appears that young Children are at the risk of

being infected with *Cryptosporidium* as compared to adults immunocompetent persons. Other workers also reported similar results (Saksirisampant *et al.*, 2002 and Rozej, 2010). The ages with high prevalence of *Cryptosporidium* may be associated with active sexual stages in both HIV positive and negative patients. At this age, females might be involved in commercial sex, day care and other poor hygienic practices. The men of this age group might indulge in outdoor activities such as alcoholic drinking, swimming which may expose them to the infection.

V. RECOMMENDATIONS

- ❖ Since HIV/AIDS is a problem in this case study area, it is recommended to extend the application of those diagnostic methods to all hospital levels.
- ❖ This study also recommends for more studies in other parts of the state

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