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Prevalence Of Cryptosporium In HIV/AIDS Patients Attending General Hospital Mubi, Adamawa State - Nigeria

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Abstract: ***The prevalence of cryptosporidium in HIV/AIDS positive patients attending General Hospital Mubi, Adamawa State, Nigeria was determined. Stool specimens from 500 HIV/AIDS patients placed on antiretroviral therapy (ART) were collected and examined for oocyst of cryptosporidium using Modified Acid-fast stain techniques (Ziehl Nelson technique). The patients' blood were collected and analyzed for CD_4^+ T-cell count using Flow-Cytometer (Paetec, GmbH, Germany). Major findings of this study include prevalence of Cryptosporidium in 189 HIV/AIDS positive patients with (37.80%). Male patients were slightly more infected than the female with the prevalence rate of 38.95% and 36.92% respectively. The age bracket of 1-10 years had (61.53%) and (55.55%) in both sexes. More so, the study showed that cryptosporidium could be related to CD_4^+ T-cell count where patients with 1-200 CD_4^+ T-cell count showed high rate of infection (78.94%) and there was significant correlation with R = (-0.74), t= 2.9 which are dependent on each other (54.8%). Treatment of infected patients, provision of toilets facilities and supply of anti-retroviral drugs are recommended for more effective management of cryptosporidium in these patients.***

Keyword: Cryptosporidium, HIV/AIDS, CD_4^+ T-cell, Prevalence.

I. INTRODUCTION

Cryptosporidium is a protozoan pathogen of the phylum Apicomplexa and it causes a diarrheal illness called crytosporidiosis (Flanigan, 1994). Cryptosporidium is zoonotic in natur (De-Silva, 2003). It does not require a vector and is capable of completing it life-cycle within a single host resulting in a microbial cyst stage which is excreted in feces and capable of infecting a new susceptible host (Flanigan, 1994). The main causes of the disease in humans *are Crytosporidium pavum* and *Cryptosporidium hominis* (previously *C.parvum* genotype 1) (Clark and Sears,1996).

Cryptosporidiosis is typically an acute –short term infection but become severe and none resolving in children and immune-compromised individuals. In humans, it may stay for up to five weeks in the lower intestine (Lindo and Lee, 2001). Infection is acquired through consumption of water, uncooked food contaminated with the feces of an infected individual or animal containing viable cysts. Some outbreak have occurred in daycare related to diaper change (De-Silva, 2003). Also hospital outbreak (Nocosomial transmission) was reported in Copenhagen where 18 HIV/AIDS positive patients were infected. The source of outbreak was from an ice-machine in the ward, contaminated by an incontinent Psychotic patient with cryptosporidiosis who uses hand to pick out ice for cold drinks (Bruce *et al*., 2000). Cryptosporidium is a common cause of severe diarrhea in immune-competent (Kuhl *et al*., 1994) as well as immune-compromised humans and domestic animals (Hunter *et al*., 1992; Hunter and Nicholas, 2002).

Human infections in healthy individuals, symptoms are self-limited and usually last less than a month. However, in HIV/AIDS patients and immune-compromised individuals diarrhea can persists for months (or even for life) and eventually become life (Clark and Sears, 1996). Cryptosporidium infection is becoming a widely recognized as a very serious public health problem which is difficult to control when outbreak occurred (Adal, 1994). Currently

cryptosporidium infection in humans has been identified in more than 60 countries in six continents (Ungar, 1990). It has been estimated in AIDS patient with diarrhea, the association of cryptosporidium ranges from 10 to 30% in the developed countries and 30 to 50% in the developing world (Petersen, 1993). Severity of cryptosporidiosis is manifested in AIDS patients with CD^+_4 T cell count below 200cell/m^2. When the number of CD^+_4 T cell count is high in AIDS patient, intestinal cryptosporidiosis will not involved, while if intestinal cryptosporidiosis is present, the CD^+_4 T cell count will be less (Lopez-Vekez *et al*., 1995). Severe weight loss (as much as 25kg) has been reported due to debilitating diarrhea (Ungar, 1990). Lack of effective therapy and resistance to available drugs is another challenge (Arora and Arora, 2010), therefore, this study is aimed at assessing the role of cryptosporidium and CD^+_4 T cell count in relation to age and sex of HIV/AIDS patient.

II. MATERIAL AND METHODS

STUDY AREA

The study was conducted in Mubi metropolis of Adamawa State Nigeria. The area comprises of two local government areas. Mubi North and Mubi South, the metropolis is located between latitudes 10^0 05' and 10^0 30'N of the equator and between longitude 13^0 12' and 13^0 19' E of the Greenwich meridian. The two local government areas occupy a land area of 192,307km^2 and support a total population of 260,009 people (National population Census, 2006). The area shares boundary with Maiha L.G.A in the South, Hong L.G.A in the West, Michika L.G.A and Cameroun Republic in the East. Mubi and its environs have a tropical wet and dry type of climate. The wet seasons runs from the months of April to October, while the dry season commences in November and end in March. The annual rainfall is about 900mm with the highest occurrence in July and August. The temperature regime in Mubi is warm to hot throughout the year because of the radiation outcome which is usually relatively evenly distributed throughout the year. However, there is usually a slightly cool period between November and February with gradual increase from January to March (Adebayo, 2004).

ETHICS

The principal Medical Officer (PMO) of Mubi General Hospital was contacted, who then introduces us to the Voluntary Counseling and Testing Centre (VCT) of the hospital. The staff of the centre duly consulted some HIV infected patients within the centre, this enable us to have an access to the required number of HIV infected patients we use to carry out this study.

SPECIMEN COLLECTION

Five hundred (500) confirmed HIV/AIDS patients with ages ranging from 1-60 years undergoing treatment in General Hospital Mubi were considered for this study. From each patient their ages, sex and nature of the stool were noted. Stool specimens were collected from each participant into a clean, wide neck, sterile bottles and about 5mls of blood specimens were collected through vein puncture using sterile syringe into a test tube containing an anti of coagulant (EDTA) for CD^+_4 T cell count. They were then transported to the laboratory within 2 hours of collection for examination.

STOOL EXAMINATION

Stool samples collected were processed using the Modified Acid fast stain technique as described by Arora and Arora, (2010). For each sample a thin smear of feces was made on a clean grease free glass slides. It was heat fixed at 70^0_C for 10 minutes and kept on the staining rack and was floaded with carbol fuchsin. The slide was heated till carbol fuchsin steamed. More carbol fuchsin was added to prevent slide from drying. The slide was then allowed to stain for 9 minutes and was washed with running tap water. The slide was decolourised with 5% aqueous sulphuric acid for 30 seconds, washed again with running tap water and counter stained with methylene blue for one minute. The slide was finally washed with running tap water, dried and covered with cover slip and examined under the microscope at x 100 magnification. The acid fast cryptosporium oocyst stained red with carbol fuchsin and non- acid- fast background stained blue.

BLOOD ANALYSIS

Blood samples were analysed for CD^+_4 T cell count using Flow Cytometry (Paetec, GmbH, Germany) as described by Wittner-Tanowitz, (1993). 20µl of CD^+_4 antibodies was placed into a paetec test tube and 20µl of blood sample was added, and mixed gently at room temperature. The mixture was agitated during incubation every five minutes for 20 minutes. 800µl of CD^+_4 buffers was added to the mixture of antibody and the sample was gently mixed. This was placed into curvet and then into a counter counting machine, the number of CD^+_4 T cell was displayed per mm^3.

STATISTICAL ANALYSIS

Data collected was analyzed using SPSS package, version 18, Chi-Square (χ^2) was used to know the level of distribution of cryptosporidium infection among patients and correlation to know the relationship between cryptosporidium infection and CD^+_4 T cell count.

III. RESULTS

The result of this study have shown that out of the 500 stool specimen screened, 189 (37.8%) were positive with cryptosporidium parasite of the positive sample, 93(38.8%) were males while 96(36.92%) were females. Table 4.1 shows that there was no significant difference between the sexes ($p>0.05$).

Gender	No. Examined	No. Infected	% Infected
Male	240	93	38.75
Female	260	96	36.92
Total	500	189	75.70

Table 4.1: Prevalence of cryptosporidium among patients examined

The result of these investigations on the prevalence of cryptosporidium infection in relation to age and sex of patients examined as shown on table 4.2 below reveal that patients with age bracket 1-10 year of both sexes had the highest prevalence of 61.53% males, 55.55% females followed by 11-20 years with prevalence of 55.88%, 39.89% respectively while 41-50 years males had the least prevalence of 25%, in females 31-40 years with prevalence of 32.75%. There was no significant correlation between age and prevalence of infection of both sex ($p>0.05$).

Gender	Male			Female		
Age	No. Examined	No. Infected	% Infected	No. Examined	No. Infected	% Infected
1-10	13	8	61.54	18	10	55.55
11-20	34	19	55.88	38	16	39.89
21-30	61	29	47.54	59	20	33.89
31-40	45	13	28.88	58	19	32.75
41-50	44	11	25.00	53	20	37.74
51-60	43	13	30.23	34	11	32.35
Total	240	93	249.06	260	96	232.17

Table 4.2: Prevalence of cryptosporidium in relation to Age and Sex of patients examined

The prevalence of cryptosporidium infection in relation to CD^+4 T-cell count in patients examined as shown in Table 4.3 have shown that patients with 1-200 CD^+4 cell count had the highest prevalence of 78.95%, followed by 201-400 CD^+4 cell count with a prevalence of 43.41% , while 1001-1200 CD^+4 cell count had the least prevalence of zero (0%).There was a significant correlation between the level of CD^+4 cell count and cryptosporidium infection ($p<0.05$, $r = -0.74$).

Range of CD^+4 T-cell count	No. Examined	No. Infected	% Infected
1-200	152	120	78.95
201-400	129	56	43.41
401-600	94	10	10.64
601-800	76	2	2.6
801-1000	46	1	2.2
1001-1200	2	0	0
1201-1400	1	0	0
1401-1600	0	0	0
1601-1800	0	0	0
Total	500	189	137.8

Table 4.3: Prevalence of Cryptosporidium infection in relation to CD^+4 T-cell count

IV. DISCUSSION

In these findings, cryptosporidium parasite was found to be highest in males (38.8%) than females (36.9%) which is in contrast to the findings of Akimbo *et al*., (2010). Generally, females tend to have high prevalence of cryptosporidium parasite than their males in HIV/AIDS patients in relation to sex (Awole *et al*., 2003) which is in contrast to present findings.

The study also revealed that cryptosporidium infection is related to ages of both sexes because it was observed that tender ages between 1-10 years had the highest rate of infection (Table 4.2). This could be as a result of children playing in and drinking contaminated water, eating raw vegetable and also lack of good nutrition and improper personal hygiene. Children with no balance diet, their immune status will be very low, thus increasing the rate of the infection.

Durack, (2001) has also reported that when CD^+4 T-cell count is low, HIV infected individuals become susceptible to a variety of opportunistic parasitic infections (especially cryptosporidium) that occur with greater frequency and severity.

In a similar study, Petersen, (1993) reported that in Ethiopia the existence of Cryptosporidium infection in children under 10 years was 30 to 50%. Also Aigbodion, (2013) reported that infection was highest between ages 20-39 in both male and female due to sexual activities not in this study. This study also shows that patients with low CD^+4 T-cell count (1-200) has the highest risk (78.95%) of being infected than those with high CD^+4 T-cell count (1001-1200). In this case, cryptosporidium is related to CD^+4 T-cell counts as it was also reported by Lopez-Velelz *et al* (1995) that as CD^+4 T-cell count increases the rate of infection decreases. Flanigan (1994) had also demonstrated that those individuals with CD^+4 T-cell count above $500/mm^3$ do spontaneously clear cryptosporidium infection while it is only patients with CD^+4 T-cell count of $140mm^3$ or less that develop chronic infection.

V. CONCLUSION AND RECOMMENDATION

From the study conducted, it can be concluded that 37.8% of patients examined that are receiving ART at the General Hospital Mubi were infected with cryptosporidium. We recommend therefore, that government and Nongovernmental organization (NGO's) should put more effort on the supply of anti- retroviral drug to HIV/AIDS patients in order to boost their immune status, since this study and other related literature have shown that cryptosporidium parasite is highly related to CD^+4 T-cell count. Also since, cryptosporidium is an opportunistic intestinal parasite that is associated with life threatening diarrhea in HIV/AIDS patient; it is recommended that early detection of this parasite for better understanding and management of diarrhea illness be carried out.

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