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RESEARCH ARTICLE

Cryptosporidiosis in relation to CD4$^+$ T- Lymphocyte counts of people living with HIV/AIDS in Jos, Plateau State, North-Central Nigeria

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ABSTRACT

This study was undertaken to determine the prevalence of cryptosporidiosis in relation to CD4$^+$ T- lymphocyte counts of people living with HIV/AIDS (PLWHA) in Jos, Plateau State, Nigeria using formol ether concentration and saturated sodium chloride floatation techniques, modified Ziehl-Neelsen staining method, history as well as clinical records of patients. A total of 280 PLWHA attending voluntary counselling and testing as well as those receiving anti-retroviral drugs at the Faith Alive Foundation were sampled for this study. Of this 66.0 (23.6%) were positive for cryptosporidiosis. Based on age group, prevalence rates were highest among 31-40 years 34.9% (23/66). This was followed by 30.2% (13/43) among age group 11-20 years, while the lowest 10.0% (6/60) was recorded among 21-30. Females recorded a higher prevalence 25.0% (35/140) than males 22.1% (31/140) which was statistically insignificant (p>0.05). Based on CD4$^+$ count, the highest prevalence of 54.8% (23/42) was observed among patients with CD4$^+$ count of ≤100. This was followed by 501-600 with 40.0% (2/5), and the lowest prevalence of 9.3% (9/97) was observed among 201-300. Patients with diarrhoea were associated with the highest prevalence of cryptosporidiosis 23.6% (66/280), while the lowest prevalence of 2.1% (6/280) was observed among patients with skin lesions. A total of 164 of the PLWHA showed one clinical sign or the other. Patients with diarrhoea recorded the highest prevalence of 83.3% (55/66) for cryptosporidiosis which was statistically significant. This was followed by patients that presented vomiting 25.0% (4/16), while the lowest prevalence 11.1% (1/9) was observed among patients with oral thrush. Patients with both weight loss and rashes recorded zero prevalence for the infection. This study confirms the opportunistic nature of *Cryptosporidium* species among PLWHA and the association of these parasites in the diarrhoea of this group of people.

Keywords: Cryptosporidiosis, *Cryptosporidium,* HIV/AIDS, CD4$^+$ T- lymphocyte count, opportunistic parasites.

1. INTRODUCTION

Cryptosporidiosis is an opportunistic and gastro-intestinal parasitic zoonoses implicated in most diarrhoea in animals and humans. The disease is self-limiting in the immunocompetent, however, it causes significant morbidity and mortality in children, old people and the immunocompromised especially People Living with HIV/AIDS.[1] The disease is caused by an apicomplexan protozoan parasite of the genus *Cryptosporidium* which affects the microvilli of the epithelial lining of the digestive tract of vertebrates.[2, 3, 4]

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Cryptosporidium is transmitted through faecal-oral route, either directly from humans to humans, humans to animals, animals to humans as well as animals to animals or indirectly through contact with water, food or air contaminated with *Cryptosporidium* oocysts.[5, 6, 7, 8]

The life cycle of each parasite includes an asexual phase of proliferation on the mucosal surface and a sexual phase of reproduction. The infective stages of both parasites are encysted when released in the faeces and capable of prolonged survival in the environment. Re-infection is possible through the ingestion of water, food, or arthropods contaminated with cysts or oocysts. The link between human and animal infections has been a question that has dominated much of the research of *Cryptosporidium*. Since organisms can be transmitted in water, the source of water contamination remains a critical issue for water authorities throughout the world.[9]

Cryptosporidiosis has been reported by several workers as an opportunistic parasite among people living with HIV/AIDS.[10, 11, 12, 13] The prevalence of HIV/AIDS in Nigeria is 3.6% implying that about 3.3 million Nigerians are living with this infection.[14] Despite this high prevalence of HIV/AIDS, most workers only reported prevalence of the disease among apparently healthy[15, 16, 17, 18, 19, 20] and people living with HIV/AIDS[11, 13] without emphasis on the $CD4^+$ T- lymphocyte counts of patients. This study was therefore designed to investigate the prevalence of cyrptosporodiosis among PLWHA in relation to $CD4^+$ T-lymphocyte counts in Jos, Plateau State, Nigeria. It is believed that the findings of this study will provide useful information on the threat pose by this parasitic zoonoses among PLWHA in relation to their $CD4^+$ T- lymphocyte counts which will help in the institution of prevention and control strategies against this infection.

2. MATERIALS AND METHODS

2.1. Study area

The study was carried out in Jos and covered two Local Government Areas in Jos metropolitan namely Jos North and Jos South. Jos is formed on a basement complex of rocks, which have produced the characteristic iceberg landscape. The Plateau highland stands at an average height of 1200 meters above the sea level. The highland is slightly undulating and rises from the escarpment of the riverside plains of the river Benue. Located in the Middle Belt zone of the country, Jos Plateau State is thus situated in the North-east area of North Central Nigeria. It lies between latitude 9'55'N and longitude 8' E of the Greenwich Meridian. The landscape is guinea savannah; mostly rocky, but with chains of hills and many captivating rock formations. The temperate climatic condition is greatly influenced by its strategic location on the Plateau, making Jos climate the nearest equivalent to the temperate climate in Europe and America. Temperature ranges from 11 ^{O}C to 30 ^{O}C with an annual rainfall of 150cm, lasting between 6 and 7 months. The months of December through February are particularly cold and dry.

2.2. Ethical issues

Ethical clearance was obtained from the management of Faith Alive Foundation before the commencement of this research. Consent forms were given to the patients to seek their permission before been sampled and only patients that agreed by signing the forms that were sampled for this study. Information on clinical signs of patients was obtained using verbal interviews and clinical records of patients.

2.3. Blood sample collection and processing for $CD4^+$ lymphocyte count

A total of 280 blood samples were collected from people living with HIV/AIDS. Samples were collected based on subjects HIV/AIDS status, $CD4^+$ T- lymphocyte counts, age, sex and clinical signs. A total of 2 ml of blood was aseptically collected between 8:00 and 11:00 am via the median cubital vein using 5 ml syringe and 21 gauge needles and transferred immediately into clean and sterile sample bottles containing K_3EDTA to prevent coagulation. To quantify $CD4^+$ T- lymphocytes, all blood samples collected were mixed and incubated in the dark for 15 minutes at room temperature. A total of 800 µl of CD4 buffer was added to the above, and read on the Cytoflow within 5 hours of collection at the Faith Alive Laboratory, using the $CD4^+$ easy count kit as described by Cassens *et al*. [21] Based on the $CD4^+$ T- lymphocyte count patients were grouped into ≤100, 101-200, 201-300, 301-400, 401-500, 501-600, >600 for the purpose of this study.

2.4. Faecal sample collection and processing

A total of 280 individuals were sampled for this study. A total of 280 stool samples each were collected from apparently healthy individuals and HIV/AIDS patients that visited the Faith Alive Foundation for HIV counselling and testing, check up and receipt of anti-retroviral drugs. Individuals were sampled based on their HIV/AIDS status, $CD4^+$ T- lymphocyte count, age group and sex. Human stool samples were collected in properly labelled sterile wide-mouthed plastic bottles and transported in Coleman icebox to the Parasitology Laboratory of the National Veterinary Research Institute, Vom, Nigeria for further analysis. Processing of samples commenced immediately but all unprocessed samples were preserved in 10% formalin solution and refrigerated till analysis was done within a week.

Faecal samples were screened for gastro-intestinal parasites using two methods namely; formol ether concentration and the saturated sodium chloride floatation techniques. The MacMaster counting technique was used for counting the frequency of occurrence of oocysts. Oocysts of *Cryptosporidium* species were

identified using smears stained with the modified Ziehl-Neelsen method following concentration by the formol ether oocysts concentration technique as described by Henriksen and Pohlenz.[22] Data collated at the end of the study were subjected to statistical analysis. Prevalence rates were calculated by dividing the number of infected individuals by the total number of individuals examined and expressed as percentages. This was done for sex, age groups, breed, and $CD4^+$ counts. Chi square test (X^2) was used to test for differences in prevalence rates of the disease based on the above variables.[23] Values of $p<0.05$ were considered significant.

3. RESULTS

A total of 280 PLWHA attending voluntary counselling and testing as well as those receiving anti-retroviral drugs at the Faith Alive Foundation were sampled for this study. Of this 66.0 (23.6%) were positive for cryptosporidiosis (Tables 1, 2 and 3). Based on age group, prevalence rates were highest among 31-40 years 34.9% (23/66). This was followed by 30.2% (13/43) among age group 11-20 years, while the lowest 10.0% (6/60) was recorded among age group 21-30 years. Prevalence rates were 29.7% (11/37), 23.3% (7/30), 11.1% (4/36) and 25.0% (2/8) for age groups 0-10, 41-50, 51-60 and <60 years respectively (Table 1). Females recorded a higher prevalence 25.0% (35/140) than males 22.1% (31/140) which was statistically insignificant (Table 1). Based on $CD4^+$ count, the highest prevalence of 54.8% (23/42) was observed among patients with $CD4^+$ count of ≤100 (Table 2). This was followed by 501-600 with 40.0% (2/5), while the lowest prevalence of 9.3% (9/97) was observed among 201-300. Also patients with $CD4^+$ count ranging from 101-200, 301-400, 401-500 and >600 recorded prevalence rates of 32.7% (18/55), 23.3% (7/30), 12.2% (5/41) and 20.0% (2/10) respectively (Table 2). Of the 164 PLWHA that presented various clinical signs, patients with diarrhoea recorded a prevalence of 83.3% (55/66). This was followed by 25.0% (4/16) for patients that presented vomiting, while the lowest was among patients with oral thrush 11.1% (1/9) as shown in Table 3. The prevalence rates of cryptosporidiosis in patients with colic, nausea and weight loss were 13.6% (3/22), 15.4% (2/13) and 5.6% (1/18) respectively (Table 3). Zero prevalence was observed among patients with both fever and rashes (Table 3).

4. DISCUSSION

Opportunistic intestinal parasites have gained serious significance in their role in causing high morbidity and mortality in humans particularly children since the emergence of HIV/AIDS.[24]

Age group (years)	Number HIV/AIDS patients examined	Number positive for Cryptosporidiosis	Prevalence (%)
0-10	37.0	11.0	29.7
11-20	43.0	13.0	30.2
21-30	60.0	6.0	10.0
31-40	66.0	23.0	34.9
41-50	30.0	7.0	23.3
51-60	36.0	4.0	11.1
>60	8.0	2.0	25.0
Total	**280.0**	**66.0**	**23.6**
Sex			
Female	140.0	35.0	25.0
Male	140.0	31.0	22.1
Total	**280.0**	**66.0**	**23.6**

SEX: $X^2 = 0.32$, P = 0.5733, Odd Ratio = 1.1720, 95% CI = 0.6744, 2.0370

Table 1: Age and sex based prevalence of cryptosporidiosis among People Living with HIV/AIDS in Jos, Plateau State

$CD4^+$ count range	Number of HIV/AIDS patients with the given $CD4^+$ count (n=280)	Number positive for Cryptosporidiosis	Prevalence (%)
≤ 100	42.0	23.0	54.8
101-200	55.0	18.0	32.7
201-300	97.0	9.0	9.3
301-400	30.0	7.0	23.3
401-500	41.0	5.0	12.2
501-600	5.0	2.0	40.0
>600	10.0	2.0	20.0
Total	**280.0**	**66.0**	**23.6**

Table 2: Prevalence of cryptosporidiosis in relation to $CD4^+$ T-lymphocyte counts of People Living with HIV/AIDS in Jos, Plateau State

Clinical signs observed among PLWHA	Number that showed the clinical sign (n=280)	Number positive for cryptosporidiosis	Prevalence (%)
Colic	22.0	3.0	13.6
Nausea	13.0	2.0	15.4
Vomiting	16.0	4.0	25.0
Pyrexia	14.0	0.0	0.0
Weight loss	18.0	1.0	5.6
Skin lesions	6.0	0.0	0.0
Oral thrush	9.0	1.0	11.1
Diarrhoea	66.0	55.0	83.3
Total	**164**	**66.0**	**23.6**

Table 3: Prevalence of cryptosporidiosis in relation to clinical signs presented by PLWHA

Cryptosporidiosis is one of the most important of these parasites implicated in protracted diarrhoea especially in the developing countries where standard sanitation and personal hygiene are lacking.[25]

Cryptosporidiosis has been reported among people living with HIV/AIDS in Nigeria[11, 13, 26,] and other parts of the world.[10, 12, 27, 28] The 23.6% overall prevalence recorded by this study greatly contrast the findings of 52.7% by Adesiji *et al*.[11] which is on the higher side and that reported by Amatya *et al*.[26] which lies on the lower side of the findings of this study. However, this finding is almost similar to the reports of Vyas *et al*.[28] who reported 25.2% of the disease in HIV positive patients with diarrhoea in Rajasthan, India. These variations may be due to differences in levels of sanitation and personal hygiene of people in these areas. Variations in locations as well as contamination of water, foodstuff and contact with animals have also been reported as possible reasons for the differences in prevalence rates of cryptosporidiosis.

The higher prevalence observed among patients with the age group 31-40 years may not be unconnected with the common habit of feeding in commercial food areas. This attitude might have increased their risk of contacting the infection as a result of poor sanitation and hygiene in such commercial food joints.

Prevalence of cryptosporidiosis accordingly to sex though not statistically significant was higher in females than males which is in accordance with the report of Dozie *et al*.[20] This might be explained by the depressed immunity associated with hormonal imbalances arising from pregnancy and lactation in females.

Relationship between $CD4^+$ T- lymphocyte counts and opportunistic parasitic infections have been reported.[13, 29, 30, 31] This study recorded higher prevalence of cryptosporidiosis among PLWHA with $CD4^+$ T- lymphocyte counts of ≤100 and 101-200 indicating higher risk of infection with opportunistic parasites in PLWHA with low $CD4^+$ T- lymphocyte counts. This finding is similar to the reports of Flanigan *et al*.[32] and Sadraei *et al*.[33] The immunodeficient state of PLWHA makes them more susceptible to cryptosporidiosis, and once established, they are not able to prevent the proliferation or clear the infecting agents.[34]

Cryptosporidiosis is usually associated with diarrhoea and weight loss.[35] This study reported 83.3% prevalence of cryptosporidiosis among PLWHA with diarrhoea. This might not be unconnected with the immunodeficient state of this group of people. This parasite has been reported in HIV/AIDS patients with diarrhoea.[11, 28] It is recommended that PLWHA should monitor their $CD4^+$ T- cell count and maintain standard hygiene and sanitation to ensure protection against *Cryptosporidium* and other opportunistic gastro-intestinal parasites.

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Conflict of Interest: None Declared

