Original Research Article

Cystoisosporiasis and Associated risk factors in HIV-infected patients with malaria in General Hospital Minna, Niger State – Nigeria.

Abstract

Background: *Cystoisospora belli* infection often occurs in immunocompriomised individuals, notably in patients suffering from HIV/AIDS. Concentration techniques were used for diagnosis of *Cystoisospora belli.*

Methods: A total of 375 samples from HIV-positive patients and 50 samples from HIV-negative patients were processed using the modified kiyoun staining methods. In addition, patient's blood samples were analysed and examined for malaria and CD4 cells by Giemsa staining technique and Flow cytometry.

Results: The overall prevalence of the coccidian was 14.93% and there was a significant association between the HIV infected patients and Non HIV subjects ($p<0.05$). There was no significant difference among the sex and age group ($p<0.05$). There was a significant relationship between Cystoisosporiasis and CD4 cell counts in HIV-positive patients ($P<0.05$). Risk factors such as level of education, swimming and occupation among HIV patients did not significantly ($p>0.05$) affect the prevalence of *C. belli* infection while source of water and contact with animals significantly affected the prevalence of *C. belli* infection. Other risks factors such as washing of hands and washing of vegetables showed significant differences.

Conclusion: This study has demonstrated that *C. belli* infection is prevalent in General Hospital Minna, Niger State, Nigeria and, as a result, may increase the burden on HIV-infected patients.

Keywords: **Cystoisosporiasis, Giemsa, Minna, CD4 cell, Cytometry**

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Background

Cystoisosporiasis which was previously known as Isosporiasis, is an uncommon diarrheal illness caused by the protozoan *Cystoisospora belli* (formerly known as *Isospora belli*). *Cystoisospora belli* was first described by Virchow in 1980. The genus *Cystoisospora* is related closely to the genera *Cryptosporidium*, *Cyclospora*, and *Toxoplasma.* However, *Cystoisospora belli* infection is not as common as infections with *Cryptosporidium*, and *Toxoplasma*. Human are the only

known host of *C. belli*, which has no known reservoir. This protozoan parasite is opportunistic in immune suppressed human hosts [1]. It primarily exists in the epithelial cells of the small intestine, and develops in the cell cytoplasm [2]. The distribution of this coccidian parasite is cosmopolitan, but is mainly found in tropical and subtropical areas of the world such as the Caribbean, Central and South. America, India, Africa, & South East Asia. In the United States, it is usually associated with HIV infection [3].

Cystoisosporiasis is found worldwide, especially in tropical and subtropical areas. Infection often occurs in immunocompromised individuals, notably in patients suffering from Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS), and outbreaks have been reported in institutionalized groups in the USA. The first documented case was in 1915. Until 2005 the etiological agent belonged to the genus *Isospora*. In 2005 it was included in the genus *Cystoisospora*. These genuses belong to different families. *Isospora* belongs to family Eimeriidae and *Cystoisospora* to Sarcocystidae. Both families belong to the suborder Eimeriorina (order Eucoccidiorida of the phylum Apicomplexa).

The causative pathogen of cystoisosporiasis is *C. belli*, a protozoan that belongs to the subclass Coccidia in the phylum Apicomplexa. The mode of transmission of Cytoisosporiasis is faecal-oral, i.e. through food or water contaminated with human faeces. In immunocompetent individuals, *C. belli* infection causes a self-limited diarrheal illness. In individuals with immunocomprise, it may cause chronic life-threatening diarrhoea and dehydration. International statistics of Endemic areas of cystoisosporiasis include Africa, Australia, the Caribbean islands, Latin America, and Southeast Asia [4]. One study found positive examination findings in up to 15% of Haitians infected with AIDS. In developing countries, 8-40% of patients with AIDS are infected. Cystoisosporiasis is the initial AIDS-defining illness in approximately 2-3% of patients with AIDS who are from Africa. Among patients with AIDS who are from South America, 10% with chronic diarrhoea have Cystoisosporiasis.

In patients with AIDS who are from Haiti and Africa, 7-20% with chronic diarrhea have cystoisosporiasis. People of all age are susceptible to *C belli* infection, although it tends to be more serious in infants and young children, possibly as a result of the risk of dehydration in the population. *C. belli* can cause severe diarrhoea in infants. No gender predilection for infection has been noted, aside from the gender distribution of people with AIDS and the risk factor most

commonly associated with this disease. No racial predilection for Cystoisosporiasis has been reported, other than racial distribution of people with AIDS in the United States.

In Immunocompetent patients, cystoisosporiasis is usually a transient, self-limited illness but can sometimes result in a protracted diarrheal illness. Cystoisosporiasis has also been reported as a contributor to malabsorption syndrome in immunocompetent patients [5]. Infection with the intestinal protozoa *Cystoisospora belli* is associated with chronic and severe diarrhoea, in particular, for persons living with AIDS and other immune-compromised individuals [6]. Infections are also seen in children and travellers to tropical regions [7, 8, 9]. Although symptoms are self-limiting in immune-competent individuals, early diagnosis and treatment can shorten the period of intestinal symptoms substantially.

Malaria is a deadly infectious disease and one of the main health problems facing developing countries in Sub-Saharan Africa (SSA) and Asia. Globally, 3.4 billion people are at risk of new malaria infections, and there are around one million deaths annually (WHO). *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* and *Plasmodium knowlesi* parasites infect humans under normal conditions [10] with *P. falciparum* and *P. vivax* being the major species that cause morbidity and mortality in children under five years of age, pregnant women and travellers from non malarious areas [11].

In Sub-Saharan African, morbidity and mortality due to malaria is decreasing despite a lack of a malaria vaccine, emergence of parasite resistance to available anti-malarial drugs, the Anopheles mosquito being resistant to insecticide residual spraying and a poor socio-economic situation that hinders malaria control and management. Efforts in drug discovery and vaccine development are hindered by limited knowledge of the underlying cellular and molecular mechanisms of host-parasite interactions during co-infection and poly-parasitism [12].

Epidemiological studies have shown that the largest burden of malaria infections is felt by communities living in poor regions of developing countries [13]. This results in co-infections, multi-parasitism or poly-parasitism [14]. In the past three decades, several studies have been undertaken to establish the nature of interaction that occurs between intestinal parasite and malaria during co-infection scenarios.

Over 40 million people are living with HIV/AIDS, the majority (more than 25 million) of whom live in sub-Saharan Africa. Up to 2.4 million deaths were recorded worldwide in 2005 [15]. People in the advanced stages of HIV infection are vulnerable to secondary infections and malignancies that are generally termed as opportunistic infections as they take the advantage of the opportunity offered by a weakened immune system [16]. In HIV-positive patients, the most clinical manifestation is chronic diarrhoea and wasting due to enteric infection [17]. Hence, this study is aimed at investigating Cystoisosporiasis and associated risk factors in HIV-infected patients with malaria in Minna, Niger state.

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MATERIALS AND METHODS

Study Area and Population

The study was conducted in Minna, Niger state which lies between latitude 3.20$_0$E and longitude 8 and 11.3$_0$N. The state occupies a land area of 76,363Km$_2$. The state is located in the Northern Central Part of Nigeria. It has an estimated population of about 3,950,249 [18]. Four hundred and twenty five (425) samples were studied comprising of 375 HIV-infected patients with malaria and Non HIV- infected subjects [17].

Sample Collection

Blood specimens were collected from each participant. Venous blood specimens were collected through the use of a dry, sterile syringe and needle; the blood was withdrawn, with minimum stasis, from a suitable vein in the arm. The blood was slowly dispensed into an ethylene diamine tetra-acetic acid (EDTA) container and mixed well [15].

Stool specimens were collected in clean wide-mouthed containers. Questionnaires were issued to the patients and subjects prior to sample collection.

Blood Analysis

1 HIV Screening

The HIV status of the patients was confirmed using HIV/AIDS test strip method for the detection of antibodies to HIV 1 and HIV 2 whole blood. 50 ml of sample (precision pipette) was applied to the sample pad (marked by the arrow symbol). One drop of buffer was then applied on the sample pad after one minute. The result was read after a minimum of 15 minutes [17].

2 Malaria Test

Malaria infection was diagnosed by examination of a stained thick blood film. Thick blood films were made from each blood sample and allowed to air dry. Slides were stained in 3% Giemsa

stain for 30 minutes, rinsed in tap water, and allowed to air dry. The stained films were examined for malaria parasites by microscopy using an oil immersion objective lens (x100). A total of 200 fields per film was examined [15, 17].

3 CD4 Count Test

The blood samples were further analyzed for CD4+ T lymphocyte cell estimation using flow cytometry (Partec Gmbh, Münster, Germany). Briefly, an amount of 20 µl CD4 PE antibody was placed into a Partec test tube and 20µl of well-mixed whole EDTA blood was added; the contents were mixed gently and incubated in the dark for 15 minutes at room temperature. This mixture was agitated during incubation every 5 minutes. Next 800µl of CD4 buffer was added to the mixture of antibody and sample and mixed gently. This was then plugged for counting [17].

Stool Analysis

The freshly void stool specimen was processed using Concentration technique and Kiyoun staining method. The concentration procedure is efficient in recovering protozoan cyst. The freshly voided stool specimens were processed using the concentration method and examined microscopically for *C. belli* and other intestinal parasites [19, 17].

About 1 gram of faeces was emulsified in 4 ml of formalin and agitated. The mixture was sieved. The mixture was spun at 2,000 rpm for four minute in centrifuge. The faecal debris on the side of the tube was detached with the aid of an applicator stick and the supernatant discarded. From this, a concentrated smear was made on a grease-free slide and stained. Homogenized fecal sample was prepared so that even amount of fecal sample was spread on slide. Fecal smear was fixed in Methanol for 3 minutes and air dried. Basic Fuschin (1%) was applied for 30 minutes and the slide was rinsed with tap water and decolorized with 1% acid alcohol for 1 minute. Malachite green (0.5%) was applied for 2 minute to counter stain and the slide was rinsed with water and air dried [17, 19].

The slide was observed under low and high power objective and the number of oocysts was observed in different fields of slide. The stained smears were examined for oocysts of *Cystoisospora belli* [20].

Data Analysis

The result obtained was put in a tabular form. Chi square(x_2) analysis was used to compare the variables. P <0.05 was considered significant.

Ethical Clearance

The ethical committee of General Hospital, Minna, Niger state gave approval for this study. Verbal informed consent of participants/subjects were sought and used in this study.

RESULTS

Prevalence of *Cystoisospora belli* infection among HIV/AIDS Patients with Malaria in General hospital Minna.

Results showed that out of 375 samples of HIV/AIDS patients collected 56 (14.93%) were positive to *Cystoisospora belli.* Also 50 samples of Non HIV/AIDS subjects were collected and they were all negative to *Cystoisospora belli* infection Therefore the prevalence of *Cystoisospora belli* among HIV patients and Non HIV subjects are 14.93% and 0.00% respectively. HIV status was a significant ($p<0.05$) factor in acquiring *Cystoisospora belli* infection (Table 1).

Table **1: Prevalence of *Cystoisospora belli* infection among HIV/AIDS Patients with Malaria in General hospital Minna.**

Samples	**No. Examined**	**No. Positive (%)**
HIV/AIDS +ve	375	56 (14.93)
HIV/AIDS –ve	**50**	0 (0.00)
Total	**425**	**56 (13.18)**

$\chi 2$ cal=8.59; χ 2; tab= 3.841; df=1 $p<0.05$

Prevalence of *Cystoisospora belli* infection among Sex group in HIV/AIDS Patients with malaria in General hospital Minna.

Table 2 showed the prevalence of *Cystoisospora belli* infection in relation to sex among HIV/AIDS Patients. Results showed that out of 90 samples examined in males, 12 were positive while 44 were positive in females out of 285 samples examined. Therefore, the prevalence of *Cystoisospora belli* infection in relation to sex among HIV/AIDS Patients are 13.33% and 15.33% for male and female, respectively. There was no significant difference in relation to sex ($p>0.05$).

Table 2: Prevalence of *Cystoisospora belli* infection among Sex group in HIV/AIDS Patients with malaria in General hospital Minna.

Sex	No. Examined	No. Positive (%)
Male	90	12 (13.33)
Female	**285**	44 (15.44)
Total	**375**	**56 (14.93)**

$\chi 2$ cal=0.21; χ 2; tab= 3.841; df=1; p>0.05

Prevalence of *Cystoisospora belli* infection among Age group in HIV/AIDS Patients with Malaria in General Hospital Minna.

The prevalence of *Cystoisospora belli* infection in relation to age group among HIV/AIDS Patients with malaria in General hospital Minna, Niger state showed that the highest prevalence for patients with *Cystoisospora belli* (20.00%) was found in the 25-29 years age group, (16.66%) was found in the 40-44 year age group, (16.12%) found in > 50 years age group, (15.51%) found in age group < 24 years, (12.67%) found in age group between 35-39 years, (12.24%) found in age group between 45-49 years and the lowest prevalence (11.86%) was found in the 30-34 year age group. There was no significant difference between the infection among the age groups (P>0.05) (Table 3).

Table 3: Prevalence of *Cystoisospora belli* infection among Age group in HIV/AIDS Patients with malaria in General Hospital, Minna.

Age group (yrs)	No. Examined	No. Positive (%)
<24	**58**	**9(15.51)**
25 – 29	**65**	**13(20.0)**
30 – 34	**59**	**7(11.86)**
35 – 39	**71**	**9(12.67)**
40 – 44	**42**	**7(16.66)**
45 – 49	**49**	**6(12.24)**
>50	**31**	**5(16.12)**
Total	**375**	**56(14.93)**

$\chi 2$ cal = 2.42; $\chi 2$ tab = 12.592; df = 6; p>0.05

***Cystoisospora belli* infection in HIV/AIDS Patients with Malaria in relation to CD4 cell Count in General Hospital, Minna.**

Cystoisospora belli infection in HIV/AIDS Patients with malaria in relation to CD4 cell Count result showed that patients with CD4 cell count below 200 had highest prevalence rate (20.51%), and lowest prevalence rate (3.00%). There was significant difference ($P<0.05$) between CD4 cell count and the infection (Table 4).

Table 4: *Cystoisospora belli* infection in HIV/AIDS Patients with malaria in relation to CD4 cell Count in General Hospital, Minna.

CD4 Count	No. Examined	No +ve (%)
< 200	**78**	**16 (20.51)**
200 – 299	**88**	**16(18.18)**
300 – 82	**12**	**12(14.63)**
400 – 499	**77**	**9(11.68)**
>500	**50**	**3(6.00)**
Total	**375**	**56(14.93)**

$\chi 2$ cal = 58.74, $\chi 2$ tab = 9.488; df = 4; $p<0.05$

Risk factors associated with *Cystoisospora belli* infection in General Hospital, Minna.

The result to evaluate the risk factors associated to *Cystoisospora belli* and environmental and personal hygiene factors are presented. Among the selected variables, source of water, swimming and contact with animals were significantly associated to the infection ($p<0.05$) while educational status, occupation, washing of hands before eating, washing of vegetables/fruits, were not significantly associated with the infection ($p>0.05$). The highest prevalence occurred in those patients who drink untreated (well, river, rain) water (34.95%) while the lowest prevalence was found in patients who wash their fruits/vegetable before eating (3.46%) (Table 5).

Table 5: **Risk factors associated with *Cystoisospora belli* infection in General Hospital, Minna.**

Risk Factors	**Yes**	**No +ve (%)**
Educational status	301	41(13.62)
No formal education	68	15(22.05)
Washing of hands		
Yes	370	46(12.43)
No	0	0(0.00)
Washing of fruits/vegetables		
Yes	289	10(3.46)
No	50	2(4.00)
Swimming		
Yes	100	4(4.0)
No	250	40(16.0)
Occupation		
Farmers	27	4(14.81)
Artisans	100	18(18.0)
Govt. Employed	85	28(17.77)
Students	85	6(7.05)
Contact with domestic animals		
Yes	88	13(14.77)
No	261	5(1.91)
Sources of water		
Untreated water (Stream/River/Well/Rain)	103	36(34.95)
Treated pipe water	272	20(7.35)

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Discussion

This is the first study to be carried out on the prevalence of *C. belli* infection and associated risk factors in HIV-infected patients with malaria in General Hospital Minna, Niger State, Nigeria.

The results obtained in this study can provide important information for future understanding of cystoisosporiasis in HIV-infected patients. The overall prevalence rate of 14.93% is higher than that found in several other studies. Akinbo *et al.* [15] observed a prevalence rate of 3.1% in Edo state, Akinbo *et al.* [20] recorded a prevalence rate of 7.8% in Benin City, Edo State, Oguntibeju. [21] observed a prevalence rate of 3.3% in Lesotho, South Africa, Assefa *et al.* [22] recorded the prevalence rate of 7.72% in Gujarat, India. The low rate of prevalence could be as a result of improved sanitation and treatment with HIV drugs. On the other hand, the prevalence rate obtained is lower compared to the study of Guptal *et al.* [23] who recorded a prevalence rate of 17.24% in Gujarat, India. The high rate of prevalence could be as a result of high diarrhea infection in the samples used [23].

It is also possible that the observed prevalence would be higher with the use of a diagnostic real time PCR assay for *C. belli*, as this is regarded as a gold standard in detecting Cystoisospora. It has been noted that with the commencement of HAART treatment in these patients a lower prevalence may also be observed. There appeared to be a significant difference in *C. belli* infection rate between HIV-infected patients and Non HIV-infected subjects ($p<0.05$). This is in agreement with work of Akinbo *et al*. [15]. This could be due to a shift in the immune status of the subjects. The prevalence of Cystoisosporiasis in relation to Age group among HIV infected patients with malaria showed that the highest prevalence (20.00%) was found in the
25-29 year age group and the lowest prevalence (11.86%) were found in the 30-34 year age group. There was no significant difference between the infection and Age group ($p>0.05$). The overall prevalence (20.00%) seen in patients between the Age of 25-29 is higher compared to the work of Akinbo *et al*. [15] who recorded the prevalence of 10.3% between the age of 31-40 age group because the groups are sexually active.

The prevalence of *Cystoisospora belli* infection in relation to sex among HIV/AIDS patients with malaria are 13.33% and 15.33% (male and female, respectively) which is higher compared to 3.3% and 3.4% (male and female, respectively) conducted by Akinbo *et al*. [15, 20]. However, both studies showed that females had the high prevalence of the infection. There was no significant difference between Cystoisosporiasis and Sex ($p>0.05$).

HIV patients with CD4+ T cells counts of <200cells/µl were at increased risk of *C. belli* infection. This higher *C. belli* infection rate may be the result of the low immune status of these

patients, which exposes them to opportunistic infections. This finding is consistent with previous reports of Akinbo *et al*. [15, 24, 25]. HIV attacks the CD4 cells that are responsible for individual immunity, thereby leading to lowered immune status. The effects of Cystoisosporiasis may lead to increases in morbidity and mortality in these patients.

The risk factors associated to *Cystoisospora belli* and environmental and personal hygiene factors are presented. The educational background of the HIV-infected patients did not significantly affect the prevalence of *Cystoisospora belli* infection without any formal education having the high prevalence (22.05%). This could be as a result of lack of awareness about the infection and personal hygiene. The occupation of the HIV-infected persons did not significantly affect the prevalence of *Cystoisospora belli* infection with Artisans having the highest prevalence (18.00%). Artisans are more likely to eat food and drink water from questionable sources as they carry out their work. They are also likely to have a poor educational background and to a large extent, poor hygiene standards. This may be the reason for the high prevalence in this group.

The source of water significantly affected the prevalence of *Cystoisospora belli* infections in HIV-infected patients having untreated (stream/rivers, well, rain, tap) water as the highest prevalence (34.95%) ($p<0.05$). Streams/rivers are not very hygienic sources of water for domestic use as a number of activities, such as bathing, defecating, and washing, are known to occur in these bodies of water. Contact with animals was a significant risk factor for acquiring *Cystoisospora belli* infection in this study ($P<0.05$) having (14.77%) as the highest prevalence.

Conclusion

This study has demonstrated that *Cystoisospora belli* infection is prevalent in General Hospital Minna, Niger State, Nigeria with a prevalence rate of 14.93% and, as a result, may increase the burden on HIV-infected patients. This study also showed that the infection were more prevalent in females than males. The study also enhances awareness of the prevalent opportunistic parasite and limits extensive evaluation or nonspecific treatment of diarrhoeal illness in HIV patients. An early and accurate diagnosis of infection will not only help in institution of specific treatment and prophylaxis (Chemoprophylaxis where ever necessary) to prevent relapse/ reoccurrence of infections in HIV patients but also in institution of various preventive measures. This will not only prolong the life of HIV infected individuals but also improve the quality of life.

Recommendations

Routine investigation of *Cystoisospora belli* infection shoud be advocated in various tertiary hospitals as this will enhance better management of HIV-infected patients. Therefore, public education program on personal hygiene, proper use of latrines, and improved sanitation should be provided to prevent and reduce the rate of protozoan infection. HAART treatment is also recommended. Further detailed investigation by using the real time PCR method should be conducted to determine the advanced detection of *Cystroisospora belli* in the area is recommended.

Availability of data and materials

Not applicable.

Consent for publication

Not applicable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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