Prevalence of intestinal parasites in relation to CD4 counts and anaemia among HIV-infected patients in Benin City, Edo State, Nigeria

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Abstract: Parasitic infections continue to take their toll on HIV positive patients by influencing the blood qualitatively and quantitatively. The objective of this study was to determine the prevalence of intestinal parasitic infections in relation to anaemia and CD4 counts among HIV-infected patients in Benin City, Nigeria. Using a serial sampling method, a total of 2000 HIV-infected patients were recruited on their first visit prior to highly active anti-retroviral therapy (HAART) at the University of Benin Teaching Hospital from August 2007 to August 2009. Stool and blood samples were collected from each patient. The stool samples were processed using the modified Ziehl-Neelsen staining technique to microscopically identify the oocysts of *Cryptosporidium* species, *Isospora belli*, *Cyclospora* species and spores of *Microsporidium* species while saline and iodine preparations were used for identifying the ova, cysts and parasites of *Ascaris lumbricoides*, hookworm, *Taenia* spp and other parasites. The blood specimens were equally analyzed using the flow cytometry for $CD4^+$ T-lymphocyte count and autoanalyzer – sysmex kx – 21 for haemoglobin concentration. The overall prevalence of anaemia was 93.3% while 18% had parasitic infections. There was a significant relationship between CD4 count <200cells/μL and anaemia (P<0.0001). *Cryptosporidium* species (P= 0.005), *A. lumbricoides* (P=0.035), hookworm and *Taenia* species (P=0.014) were associated with anaemia. Anaemia was associated with CD4 count while *Cryptosporidium* species, *Ascaris lumbricoides*, hookworm and Taenia species were the intestinal parasitic agents associated with anaemia. In conclusion the prevalence of anaemia in HIV-infected patients is high low CD4 count is a significant risk factor of acquiring anaemia. Except for isosporiasis, cryptosporidiosis, *A. lumbricoides*, hookworm and *Taenia* species in HIV infected individuals are parasitic agents associated with anaemia. Routine screening for intestinal parasites and holistic management of anaemia is advocated.

Keywords: intestinal parasites, anaemia, CD4 counts, HIV/AIDS, Nigeria

Introduction

Parasitic infections continue to take their toll on HIV-infected individuals by negatively influencing the blood qualitatively and quantitatively. Anaemia, which can be mild to severe, acute or chronic, is one of the multitudes of complications associated with parasitic infections (Agiomea, 2003). Anaemia is a frequent complication of HIV/AIDS which contributes to reduced quality of life and increased morbidity and mortality (Akinbo *et al*., 2009a). The relationship between parasitic infection and anaemia is a pathogeno-physiologic type (Stephenson *et al*., 1985) where it is recognized that certain factors play important roles. These include the strain and number of the parasites, the size and site, metabolic processes of the parasite, age and level of immunity at the time of infestation, presence of co-existing diseases or

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conditions which reduce immune responses, malnutrition, and the life style of the person infested (Cheesebrough, 1999).

In Africa, intestinal parasitic infections are among the major causes of anaemia due to generally poor standard of living among the populace (Latham *et al*., 1977; Mupfasoni *et al*., 2009). Previous studies have revealed that HIV patients with malaria infection are more likely to develop anaemia, while a $CD4^+$T cell count of <200cells/μL was associated with an increased risk of parasitic infection among HIV-infected patients (Akinbo *et al*., 2009b). There is paucity of information as regards an association of anaemia and CD4 count among HIV-positive subjects that are infected with parasitic infections. Against this background, this was study carried out to determine the prevalence of intestinal parasitic infections in relation to anaemia and CD4 counts among HIV-infected patients in Benin City, Nigeria.

Materials and Methods

Study area and population

The study was carried out in the University of Benin Teaching Hospital, Benin City, Nigeria. It is located in the South-South geopolitical zone of Nigeria. It serves as a referral hospital to about 6–10 States in Nigeria. It is a centre for Institute of Human Virology, Nigeria and President's Emergency Plan for AIDS Relief (PEPFAR) HIV/AIDS interventions in the zone.

HIV- positive patients attending HIV clinic at the University of Benin Teaching Hospital, Benin City, Nigeria from August 2007 to August 2009, were recruited for this study. Patients on HAART, antiparasitic agents and AIDS defining conditions were excluded from the study. The study participants were selected by serial sampling technique.

Specimen collection and processing

Stool and 5ml of blood specimens were collected from each patient. The stool specimens were collected in clean wide-mouthed container while 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 ethylene diamine tetra-acetic acid (EDTA) container and mixed well (Baker *et al*., 2001).

Freshly voided stool specimens were processed using formol-ether concentration method and examined microscopically for intestinal parasites as previously described (Cheesbrough, 1999). Part of the stool specimen was preserved in 10% formal-saline. From this, a concentrated smear was made, fixed with methanol and stained by a modified Ziehl-Neelsen stain. This was used to detect oocyst of *Cryptosporidium* species, *Isospora belli, Cyclospora* species and spores of *Microsporidium* species.

The blood specimens were used for $CD4^+$ T-lymphocyte count and haemoglobin concentration. The $CD4^+$ count was determined by flow cytometry (Partec, Gmbh, Germany) while haemoglobin concentration was determined using an autoanalyser-sysmex kx-21 (Sysmex Corporation, Kohe, Japan). Anaemia was defined according to WHO criteria (Bentler & Wealen, 2006). For males, this was haemoglobin concentration <13g/dl and for females it was <12g/dl.

Data analysis

Data were analyzed using Chi-square (X^2) test (for differences in prevalence) and odd ratio analysis (to assess risk factors). The statistical software INSTAT® was used for the analysis.

Ethical consideration

The Ethical Committee of the University of Benin Teaching Hospital, Benin City, Nigeria, approved the protocol of this study. Verbal informed consent was obtained from every patients enrolled in this study.

Results

A total of 2000 patients were recruited. They consisted of 668 (33.4%) males and 1332 (66.6%) of females with age ranging from 21-70years (37.06 ± 9.19). The majority (1866; 93:30%) of HIV patients had anaemia while only a few (18.0%; N=360) had parasitic infections. CD4 count <200cells/μL was a significant risk factor for acquiring anaemia (OR=42.65, 95% CI = 2.64, 688.00; *P*<0.0001) (Table 1).

Table 1: Relationship between anaemia, parasitic infections and CD4 counts

Characteristics		CD4 Count (Cells/ul)		OR	95%CI	P-value
		<200 (%)	≥200 (%)			
Anaemia	Yes	255 (100)	1611 (92.3)	42.65	2.64, 5.88.0	
	No	0 (0)	134 (7.68)	0.02	0.0, 0.38	<0.0001
Infection with	Yes	79 (100)	233 (82.92)	33.03	2.01, 542.2	
anaemia	No	0 (0.0)	48 (17.08)	0.03	0.00, 0.50	0.0002

Isospora belli and *Cryptosporidium* species were the only intestinal parasite associated with CD4 count <200cells/μL (Table 2). *Cryptosporidium* species, *Ascaris lumbricoides*, hookworm and *Taenia* species were the intestinal parasites associated with anaemia (Table 3). The prevalence of parasites were *Entamoeba histolytica* (3.33%), *Giardia intestinalis* (0.56%), *Isospora belli* (7.78%), *Cryptosporidium* species (22.22%), *Ascaris lumbricoides* (33.06%), Hookworm (20.56%), *Strongyloides stercoralis* (6.39%), *Trichuris trichuria* (5.00%) and *Taenia* species (1.11%).

Table 2: Effect of CD4 count on the prevalence of parasitic agents

Parasitic Agents	CD4 count (cells/μL)			95%CI	P-value
	< 200 n= 255*	≥ 200 n=1745	OR		
Entamoeba histolytica	0	12	0.271	0.016, 4.601	0.371
Giardia intestinalis	0	2	1.365	0.065, 28.530	0.589
Isospora belli	11	16	4.872	2.234, 10.622	<0.0001
Cryptosporidium species	59	21	24.712	14.698, 41.551	<0.0001
Ascaris lumbricoides	16	103	1.067	0.619, 1.839	0.926
Hookworm	9	65	0.946	0.465, 1.923	0.877
Strongyloides stercoralis	3	20	1.027	0.303, 3.481	0.966

Trichuris trichiura	4	15	1.838	0.605, 5.583	0.457
Taenia species	1	3	3.431	0.310, 37.998	0.839

Key: * number of patients with CD4<200 cells/µl; ** number of patients with CD4 ≥200 cells/µl. Figures in columns 2 and 3 are the number of patients infected with a particular parasitic agent

Table 3: Association of parasitic agents with anaemia

Parasite	No. with anaemia (%) N=1866	No. without anaemia (%) N=143	OR	95% CI	P-value
Entamoeba histolytica	12	0	1.813	0.107, 30.811	0.725
Giardia intestinalis	2	0	0.361	0.017, 7.557	0.705
Isospora belli	28	0	4.170	0.253, 63.724	0.295
Cryptosporidium spp.	80	0	12.121	0.747, 196.680	0.027
Ascaris lumbricoides	119	0	18.395	1.137, 297.65	0.005
Hookworm	74	0	11.180	0.689, 181.53	0.035
Strongyloides stercoralis	23	0	3.429	0.207, 56.803	0.383
Trichuris trichiura	18	0	2.692	0.161, 44.948	0.504
Taenia species	2	0	0.071	0.010, 0.507	0.014

Discussion

Anaemia is one of the haematological complications seen in HIV-positive patients (Moyle, 2002) and is a significant predictor of progression to AIDS or death (Odunukwe *et al*., 2005). Parasitic infections are known to cause anaemia among non-HIV subjects (Oguntibeju, 2003; Agbolade *et al*., 2009) as well as among HIV patients (Akinbo *et al*., 2009a). As HIV infection progresses to AIDS, a number of opportunistic infections and other sequela occur. Parasitic infections (especially intestinal) and anaemia are among the morbidities seen in HIV patients (Oguntibeju, 2006; Zelalem *et al*., 2008; Akinbo *et al*., 2009a, 2010).

The prevalence of HIV-positive patients who had intestinal parasitic infections in this study was lower than the previous reports by Oguntibeju (2006). The difference is due to type of patients used. Oguntibeju (2006) used both HIV-positive and AIDS patients, while only HIV-positive patients were used in this study. Anaemia prevalence in this study was higher than previous reports in HIV patients (Akinbo *et al*., 2009a; Omoregie & Eghafona, 2009; Omoregie *et al*., 2009). The cause of anaemia in HIV-positive patients is multi-factorial and includes infections, neoplasm, dietary deficiencies, blood loss, medications and antibodies to antiretroviral agents (Moyle, 2002; Omoregie *et al*., 2008). Also, bone marrow suppression, especially the erythroid lines, by the AIDS virus is known to result in anaemia (Odunukwe *et al*., 2005). Indeed, CD4 count of <200cells/µL was a significant risk factor for anaemia in this

study. This agrees with a previous report by Curkendall *et al*. (2006). However, according to Omoregie *et al.* (2009) the relationship between CD4 counts and anaemia depends of the cut-off values for CD4.

In a similar vein, intestinal parasitic infection among HIV patients with anaemia is significantly associated with CD4 count <200cells/μL. Intestinal parasitic infections contribute to the anaemia seen in HIV-positive patients, especially, when the CD4 count is < 200cells/ μL. This is important as CD4 count <200cells/ μL is believed to be associated with disease progression and opportunistic infections (Oguntibeju *et al*., 2006). This may explain the association between CD4 count and parasitic infections among HIV patients with anaemia.

The parasites observed in this study had previously been noted in HIV patients (Hailermariam *et al*., 2004; Oguntibeju, 2006; Kwitshana *et al*., 2008). *I. belli* and *Cryptosporidium* species were the only parasites associated with CD4 count < 200cells/ μL. This is in agreement with other recent observations (Akinbo *et al*., 2009b; Akinbo *et al*., 2010). Isosporiasis and cryptosporidiosis are opportunistic infections (Zelelam *et al*., 2008; Guptal *et al*., 2008). In relation to anaemia, *Cryptosporidium* species, *A. lumbricoides*, hookworm and *Taenia* species were the only parasites that were significantly associated with anaemia. *A. lumbricoides*, hookworm and *Taenia* species are known to cause anaemia among non-HIV subjects (Oguntibeju, 2003; Vuylsteke *et al*., 2004; Agbolade *et al*., 2009). To our knowledge, this is the first report associating cryptosporidiosis with anaemia. Cryptosporidiosis is usually associated with diarrhea and weight loss (Kulkarni *et al*., 2009). This will require further investigation especially the mechanism by which *Cryptosporidium* species cause anaemia. It is important that HIV-positive patients be screened for intestinal parasitic infections so that there can be a holistic management of anaemia when indicated, in order to improve quality of life of HIV-positive patients.

In conclusion the prevalence of anaemia in HIV-infected patients is high low CD4 count is a significant risk factor of acquiring anaemia. Isosporiasis and cryptosporidiosis were associated with immunodeficiency while Cryptosporidium species, *Ascaris lumbricoides* and hookworm were the intestinal parasitic agents associated with anaemia

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We acknowledge with thanks the Management of University of Benin Teaching Hospital for permission to carry out this study.

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