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Research Article

EVALUATION OF INTESTINAL PARASITE LOADS, HAEMOGLOBIN LEVELS AND CD4 COUNTS OF PATIENTS ON ANTIRETROVIRAL COMBINATION THERAPY (cART) IN CALABAR METROPOLIS, NIGERIA

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ABSTRACT

This was a case-control survey on the evaluation of intestinal parasite loads, haemoglobin levels and CD4 counts of patients on antiretroviral combination therapy (cART) in Calabar, Nigeria.

Methods: Stool samples were collected from case-control study of 500 participants with 350 people living with HIV on cART and 150 HIV negative participants and examined using direct microscopy, formol-ether concentration technique and modified Ziehl-Neelsen technique; Human Immunodeficiency virus status was determined using serial HIV testing algorithm; Haemoglobin levels were determined using cyanmethaemoglobin method; CD4 count was analyzed using PartecCyFlow counter.

Results: Among the test subjects, 11.14 (39/350) were infected with enteric parasites, mean helminth ova count was 494.36+37.50 ova/g with mean haemoglobin level of 10.24+0.28 g/dl. *Entamaoeba histolytica* (31%) was the commonest protozoan parasite, *Ascaris lumbricoides* (15%) was the commonest helminth parasite and *Cryptosporidium parvum* (23%) was the commonest opportunistic parasite.

Conclusion: It is recommended that strategies aimed at diagnosing and treating for intestinal parasite infections, monitoring and improving the haemoglobin levels of people living with HIV be encouraged.

Keywords: Prevalence; intestinal parasites; haemoglobin level; HIV; Calabar; Nigeria

INTRODUCTION

The human immunodeficiency virus (HIV) infection is a serious challenge to global health. About 4.5 million infected people live in Nigeria [1]. HIV/AIDS and intestinal parasitic infections are linked in a viscous circle [2]. Intestinal parasitic infections are among the most common infections worldwide and about 3.5 billion persons, mostly children, are estimated to be infected. Reports have shown that these infections frequently present as diarrhoea and significantly, the infections have been recorded in 50% to 90% of HIV/AIDS cases with 90% prevalence rate reported in Africa [5]. Although low haemoglobin is one of the haematological defects present in people living with HIV [5], intestinal parasitic infections are reported to commonly cause low haemoglobin in HIV/AIDS individuals [6,7] thereby endangering the lives of people living with HIV/AIDS [8,9].

In Nigeria, intestinal parasitic infection constitutes a major public health challenge [10]. Illiteracy, absence of clean drinking water and poverty has been shown to promote infection with intestinal parasites [11] and these have been reported as driving forces for HIV infection [12]. The intestinal parasites frequently encountered in HIV co-infection includes *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Entamoeba histolytica*, *Giardia intestinalis*, *Trichuris trichiura* and hookworms which have association with diarrhoea and iron deficiency anaemia while *Isospora belli* and *Cryptosporidium parvum* are the most common opportunistic intestinal parasites found in people living with HIV [13]. This study was to evaluate of the intestinal parasite loads, haemoglobin levels and CD4 counts of patients on antiretroviral combination therapy (cART) in Calabar Metropolis, Nigeria.

MATERIAL AND METHOD

Study area

The area of study is Calabar, the capital of Cross River State in South-South region of

Nigeria. Administratively, the city is made up of Calabar Municipal and Calabar South Local Government Areas.

Study design

This was a case-control study of 500 participants with 350 people living with HIV on cART and 150 HIV negative participants to evaluate the intestinal parasite loads, haemoglobin levels and CD4 counts of patients on antiretroviral combination therapy (cART) who visited General Hospital Calabar, Lawrence Henshaw Memorial Hospital, Calabar, Primary Health Centre, Ekpo Abasi, Calabar and FHI 360 in Calabar through continuous sampling and testing of consented participants. Participants who were on specific anti-enteric helminthic drugs and haematinics in the last two weeks preceding specimen collection were excluded from the study. This was to avoid the destruction of the potential enteric parasites and the increase in the erythrocyte levels of the consented participants. The participants were non-diahorric.

Samples collection and processing

Stool collection

Stool samples were collected from both the test participants into a sterile, sealed universal container given to each of the participants and transported immediately to the Microbiology and Parasitology unit of the University of Calabar Teaching Hospital laboratory, Calabar, Nigeria for analysis.

Macroscopic examination of stool

Stool samples were examined macroscopically in the laboratory to check for colour, consistency and presence of blood, mucus and worms in the stool samples.

Microscopic examination of stool

Identification of parasite species was done using microscopic examination of direct smear and smear with iodine and formol-ether concentration method.

Wet preparation

A small quantity of the sample was used and homogenized with few drops of physiological saline. Smear was prepared on a grease-free slide using a drop of the homogenized mixture. On another slide, smear was prepared from the homogenized mixture. A drop of physiological saline and a drop of iodine were added to the first and second slides respectively.

A cover slip was placed on each sample on the slide and was examined under the microscope with X10 and X40 objective lenses respectively [14].

Formol-ether concentration technique

In order to concentrate eggs, cysts and trophozoites with low number in stool samples which could have been missed using wet preparation, formol-ether concentration technique was used. A small quantity of the sample was emulsified in 10ml of distilled water, allowed to sediment and separated using two layers of gauze in a funnel. The filtrate was centrifuged at 2000rpm for 2 minutes and the supernatant was discarded. The sediment was resuspended in 10ml of physiological saline and spin. The supernatant was again discarded and the sediment was suspended in 7ml of 10% formol-saline and allowed to stand for 10 minutes for fixation. Diethyl ether of 3ml was added to the tube containing the mixture, corked and vigorously shook. The cork was removed and centrifuged at 500rpm for 3 minutes. The mixture formed four layers: a top layer of ether, a plug of debris at the interface, the formol saline layer and the sediment at the bottom. The debris was carefully removed from the sides of the tube using glass rod and the top three layers were discarded. The sediment was suspended in few drops of formol-saline, placed on a clean grease-free slide, covered with a coverslip and examined microscopically as wet mount and iodine mount [14].

Modified Ziehl-Neelsen technique

This was carried out to identify the oocysts of *Cryptosporidium* species and *Cyclospora cayetanensis*. Smear was prepared from the formal ether concentration technique sediment. The smear was air-dried and fixed using methanol for 2 minutes. The smear was stained with carbol fuchsin (unheated) for 15 minutes; the stain was washed off with water; the smear was decolorized with 1% acid alcohol for 10 seconds and finally washed off with water. The slide was counter stained using methylene blue for 30 seconds, washed off with water and the slide was allowed to air dry, kept on a draining rack. Microscopic examination was carried out with a lower power magnification for the detection of oocysts and the oil immersion objective lens for identification [14].

Blood collection

Five milliliter (5ml) of blood samples were also collected from the participants into EDTA container for algorithm of HIV testing and the determination of haemoglobin level.

Algorithm of HIV testing

HIV screening was done using the serial algorithm of screening with determine and confirmed result with Unigold as described by [15].

Determination of haemoglobin level

Cyanmethaemoglobin method was used to determine the haemoglobin level of the patients and this was done as described by [16].

Analysis of whole blood for CD4 cell count

CD4 count was analyzed using Partec CyFlow counter made in Germany. Briefly, 20µl fresh whole EDTA blood was added to 20µl CD4/MAb in a test tube and incubated in the dark at room temperature for 15 minutes and 800µl of buffer was added, connected to the CyFlow and allowed to run. Automatically generated report of the blood sample was displayed on the screen of the machine [17].

Socio-demographical data collection

Questionnaires were administered to the participants to obtain their socio-demographic data. Relevant data were also collected by inspecting the participants' medical records.

Statistical analysis

Data collected were analyzed using SPSS Statistics 20 manufactured by International Business Machines (IBM) Corporations. The difference in the proportion of infection of the participants was determined by Chi square and P-value <0.05 was considered significant.

RESULTS

Table 1 presents the prevalence of intestinal parasite infections and haemoglobin levels according to age. Out of the 350 examined people living with HIV (test subjects), 11.14% (39/350) was infected with intestinal parasites, mean helminth ova count was 494.36±37.50 ova/g and mean haemoglobin level was 10.24±0.28 g/dl. Subjects, aged 10-19 years had highest percentage of intestinal parasite infection of 47.37% (9/19) were recorded among the age group of while the least percentage of intestinal parasite infections of 5.33% (4/75) were recorded among the age group of 40-49 years.

Test subjects in the age group of 20-29 years recorded the highest mean helminth ova count of 525.0±655.11 ova/g of stool while those in the age group of >50 years recorded the lowest mean helminth ova count of 430.13±264.58 ova/g of stool. The highest mean haemoglobin level of 10.54±0.98 g/dl was recorded among the age group of >50 years while the least haemoglobin level of 9.9±1.51 g/dl was recorded among the age group of 20-29 years. There was no statistically significant difference in infection according to age (X^2=32.13, df=4, P<0.05).

Table 1: Prevalence of enteric parasites infection and Hb levels among the participants based on age

Age (in years)	Test (HIV positive subjects) n = 350				Control (HIV negative subjects) n = 150			
	No. examined	No. (%) with enteric parasites	Mean helminth ova counts ± SD (ova/g)	Mean Hb levels ± SD (g/dl)	No. examined	No. (%) with enteric parasites	Mean helminth ova counts ± SD (ova/g)	Mean Hb levels ± SD (g/dl)
10–19	19	9 (47.37)	500.0 ± 458.26	10.0 ± 1.15	38	3 (7.89)	5 66.67 ± 378.59	10.52 ± 2.20
20–29	72	4 (5.55)	525.0 ± 0	9.9 ± 1.51	38	3 (7.89)	300.0 ± 264.58	11.21 ± 1.21
30–39	109	16 (14.68)	516.67 ± 306.05	10.45 ± 1.36	46	2 (4.35)	400.0 ± 282.84	10.98 ± 1.68
40– 49	75	4 (5.33)	500.0 ± 0	10.32 ± 1.33	28	2 (7.14)	200.0 ± 141.42	11.79 ± 1.01
≥50	75	6 (8.0)	430.13 ± 264.58	10.54 ± 0.98	8	3 (37.5)	466.0 ± 0	11.54 ± 1.53
Total	350	39 (11.14)	494.36 ± 37.50	10.24 ± 0.28	150	13 (8.67)	386.73 ± 142.70	11.21 ± 0.49

P-value: 0.05 , df:4, X^2:32.13

The prevalence of intestinal parasite infections and haemoglobin levels among the subjects based on gender is shown on Fig. 1. The female test subjects had a lower infection percentage of 10.46% (25/228) than 11.48% (14/122) among the males.

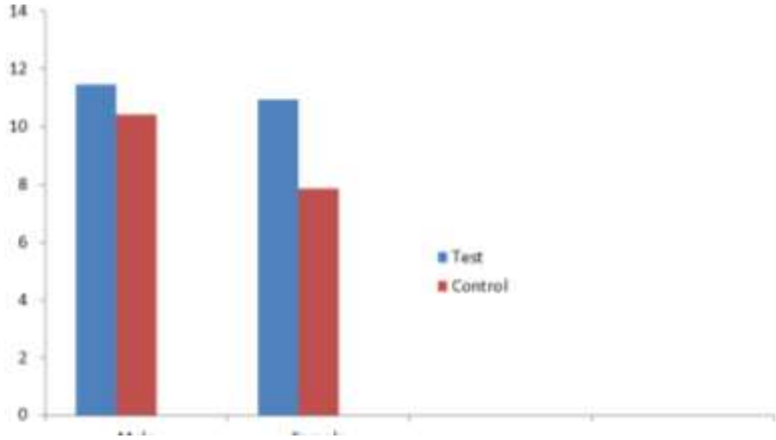

Fig. 1: Prevalence of intestinal parasites in patients on antiretroviral drug according to gender

Table 2 presents the prevalence of intestinal parasite infections and haemoglobin levels among the test based on CD4 count. Out of the 350 test subjects, the highest infection percentage of 16.13% (10/62) was recorded among those with CD4 <200 cells/µl while the least intestinal parasite infections percentage of 9.76% (4/41) were recorded among those with CD4 601-800 cells/µl. The highest mean helminth ova count of 624.98±391.58 ova/g of stool was observed among those with CD4 401-600 cells/µl while the least mean helminth ova count of 275.0±190.86 ova/g of stool was observed among those with CD4 >800 cells/µl. Those with CD4 400-601 cells/µl had the highest mean haemoglobin levels of 10.57±1.23 g/dl while those with CD4 401-600 cells/µl had the lowest mean haemoglobin level of 10.01±1.50 g/dl.

Table 2: Prevalence of enteric parasite infections and Hb levels among the test subjects based on CD4 count

Test (HIV positive subjects)n = 350				
CD4 counts (cell/µl)	**No. examined**	**No. (%) with enteric parasites**	**Mean helminth ova counts ± SD (ova/g)**	**Mean Hb levels ± SD (g/dl)**
≤ 200	62	10 (16.13)	600.0 ± 565.689	10.36 ± 1.25
201-400	151	15 (9.93)	571.82 ± 550.76	10.07 ± 1.14
401-600	76	8 (10.53)	624.98 ± 391.58	10.57 ± 1.23
601-800	41	4 (9.76)	400.0 ± 282.84	10.01 ± 1.50
≥ 801	20	2 (10.0)	275.0 ± 190.86	10.19 ± 1.59
Total	**350**	**39 (11.14)**	**494.36 ± 37.50**	**10.24 ± 0.28**

Fig. 2 presents the distribution of intestinal parasites among the test subjects. The common intestinal parasites detected among the test subjects. Out of 350 test subjects, 39 intestinal parasites were found. The intestinal protozoan parasites in the study were *Entamoeba histolytica*, 31% (12/39), the opportunistic protozoan were *Cryptosporidium parvum*, 23% (9/39), and *Isospora beli*, 5% (2/39) while the intestinal helminth parasites were *Ascaris* lumbricoides, 15% (6/39), *Strongyloides stercoralis* 8% (3/39), hookworm, 10% (4/39), *Trichuiris trichuiria,* 5% (2/39) and *Taenia* species, 3% (1/39).

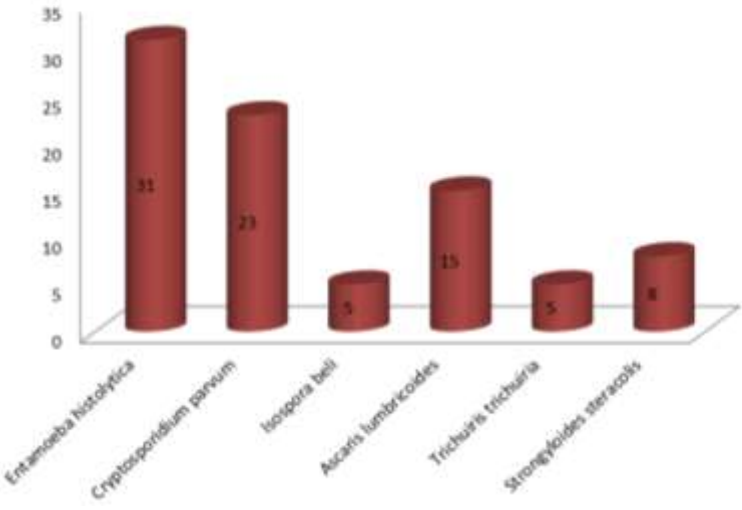

Fig. 2: Distribution of intestinal parasites among the test subjects

DISCUSSION AND CONCLUSION

Low haemoglobin level is one of the haematological defects present in people living with HIV [5] and parasitic infections are reported to cause anaemia both in HIV negative and HIV positive persons [6,7]. However, HIV persons are of more concern because intestinal parasites and low haemoglobin levels are one of the leading causes of progression to AIDS and morbidity in HIV patients [8,9].

This research was designed to determine the prevalence of intestinal parasite loads and haemoglobin levels of people living with HIV in Calabar metropolis, Nigeria. The prevalence of intestinal parasites among the test subjects in this study was 11.14% (39/350). This is lower than 29% previously reported by Inyang-Etoh*et al.* [18] in Calabar, 15.3% by Akinbo *et al.* [19] in Benin and 8.7% by Etok *et al.* [20]. The reason for this low prevalence of intestinal parasites may have been due to the socio-economic status and hygienic levels of the study subjects. High level of awareness among this group of persons on the dangers posed by parasites (particularly intestinal) to their health status and the need for periodic treatment with anti-parasitic therapies may have accounted for the low intestinal parasite prevalence in this study. The conduct of the study in dissimilar areas of urban and sub-urban settlements could enhance the inconsistency of the findings as seasonal variation and endemicity in the area of study could also cause the differences in the prevalence of the parasites.

The haemoglobin level of the test subjects was 10.24+0.28 g/dl lower than those of the control subjects with mean haemoglobin level of 11.21+0.49 g/dl in this study. This may have been due to the fact that anaemia is a multifactorial condition which may have been attributed to effects of infections, malignancy, malnutrition and polypharmacy. Gedefaw*et al.* [21] has attributed cART regimen and the duration of cART as anaemia predictors in cART adherent individuals and this might be due to AZT toxicity. Most of the study subjects in this study were on a first line of combination therapy like lamten EFV (TDF/3TC/EFV), combi pack (AZT/3TC/NVP) and second line of combination therapy like lamten alluvia (TDF/3TC/LPV/r), combivir alluvia (AZT/3TC/LPV/r) and lamten atazauavir (TDF/3TC/ATV/r). Most of these drugs were in combination with 3TC and AZT which have been associated with inducing anemia [22,23]. WHO [24] had reported that 6,650,000 patients who were on treatment with AZT in 2010 were anaemic.

Test subjects with CD4 <200 cells/µl had the highest percentage of intestinal parasites of 16.13% (10/62). This was in tandem with Amatya *et al.* [25] who noted that intestinal parasites were highest in those with CD4 <200 cells/µl but differed with Abdullahi *et al.* [26] who reported highest prevalence of intestinal parasites in those with CD4 200-350 cells/µl. The occurrence of intestinal parasites in this group of persons could have been due to the progressively suppressed immune systems thereby making them prone to infections.

Persons between the ages of 30-39 years had the highest percentage of HIV cases of 31.14% (109/350) in the study. This could have been attributed to high sexual activeness and economic dependence among the members of this group and also accounted for 4.57% of the intestinal parasite infections in this study and the infection was statistically significant according to age (P<0.05). This was in tandem with UNAIDS [27] who reported that persons at 25+ years are responsible for the distribution of most of the new HIV infections in sub-Saharan Africa. Those in the age group of 10-19 years accounted for the highest percentage of intestinal parasite infections (47.36%) in this study. This was in contrast with Kipyegen *et al.* [28] who reported that persons between 20-39 years recorded the highest prevalence of intestinal parasites.

Female participants were observed to be the highest in the study with 66% (228/350) against 34% (122/350) of the male participants. Women are reported to be the most susceptible groups to infections and they have accounted for 120,003 new HIV cases in Nigeria in 2013 [29]. The females, however, accounted for the least percentage of intestinal parasites with 10.96% (25/228) and the infection was statistically significant between gender (P<0.05). This agreed with that of Akinbo*et al.* [19] who reported a higher percentage of intestinal parasite infections in females and inconsistent with that of Inyang-Etoh*et al.* [18] who reported a higher prevalence among females.

The rate of occurrence of intestinal protozoan parasites in this study was 59% (23/39) higher than that of helminths, 41% (16/39) in this study. This observation was similar to that of Inyang-Etoh *et al.* [18] in Calabar, Nigeria, Kipyegen *et al.* [28] in Kenya and Tian*et al.* [30] in China who reported that the rate of occurrence of intestinal protozoan parasites was the highest among intestinal parasite infections. *Entamoeba histolytica* were the commonest intestinal parasites in this study with 31% (12/39). This agreed with Abaver *et al.* [31] in Abuja and Jegede *et al.* [32] in Kano who reported that *Entamoeba histolytica* were the commonest intestinal protozoan parasites. *Ascaris lumbricoides* was the commonest helminth parasites in this study with 15% (6/39). This confounded with Kipyegen *et al.* [28] who reported *Ascaris lumbricoides* as the commonest helminth in Kenya. *Crytosporidium parvum* were the commonest opportunistic intestinal parasites in this study with 23% (9/39). This was similar with reports of Akinbo *et al.* [19] in Benin and Amatya *et al.* [25] in Nepal.

It is recommended that strategies aimed at diagnosing and treating intestinal parasite infections and improving the haemoglobin levels of people living with HIV be encouraged.

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Conflict of interest: Not declared

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