Asian Journal of Research and Reports in Gastroenterology

3(2): 32-38, 2020; Article no.AJRRGA.58260

The Clinical Effects of Intestinal Parasites and Malarial on People Living with HIV/AIDS within Makurdi Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TOM and OA designed the study, author VUO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TOM and OA managed the analyses of the study. Authors VUO and TOM managed the literature searches. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. John K. Triantafillidis, IASO General Hospital, Greece.
Reviewers:
(1) M. Rout, ICAR-DFMD, India.
(2) A. B. Hameed Raina, Tata Hospital Mumbai, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/58260

Original Research Article

Received 06 April 2020
Accepted 12 June 2020
Published 16 July 2020

ABSTRACT

Parasitic infection has been implicated to have some effects on the packed cell volume, white blood cell count and CD4 counts of the infected person. This research was conducted to determine the effect of intestinal parasites and Malaria on some blood parameters of people living with HIV/AIDS within Makurdi metropolis, Benue State, Nigeria. Four hundred (400) blood and stool samples of people living with HIV/AIDS were collected from 45 NAF Base Hospital Makurdi and Bishop Murray Hospital Makurdi and examined for intestinal parasites and malaria infections between the months of September and December 2014. Formal-ether concentration technique was used for the stool examination; the thick film was prepared for blood examinations. The packed cell volume, white blood cell count and the CD4 count of the sampled population were also determined respectively. The parasitic infections found was Malaria *(Plasmodium falciparum)* (46%).This was followed by Taeniasis (*Taenia solium)* (13%), Amoebiasis (*Entamoeba histolytica)*

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(8%) and Hookworm disease (4%). The Packed Cell Volume, White Blood Cell Count and CD4 determined were compared with the World Health Organization standard. Packed Cell Volume (χ^2 Cal= 111.407 df 3, P<0.05), White Blood Cell Count (χ^2 Cal= 121.662 df 3, P<0.05) and CD4 count (χ^2 Cal= 175.311 df 2, P<0.05). The prevalence rate of intestinal parasite and Malaria is high among people living with HIV/AIDS within Makurdi Metropolis.

Keywords: Packed cell volume; white blood cell count; CD4; malaria; intestinal parasites.

1. INTRODUCTION

Intestinal parasitic infections play an important role in the progression of HIV infection, by further disturbing the immune system while it is already engaged in the fight against HIV [1]. The gastrointestinal pathology associated with HIV infection comprises significant enteropathy with increased levels of inflammation and decreased levels of mucosal repair and regeneration (Douek and Brenchley, [2]. The pathogenic intestinal parasites such as *Cryptosporidium, Cyclospora, E. histolytica* and *Giardia*, can last for months in patients with AIDS, causing malabsorption of nutrients, gradual debilitation through dehydration, and metabolic abnormalities and are responsible for severe diarrhoea episodes [3,4].

HIV infection leads to loss of $CD4^+$T cells, which leaves affected individuals mortally susceptible to opportunistic infections [5]. Many of the opportunistic infections that ultimately plague such individuals involve infectious agents that are normally checked by the mucosal barriers which include *Cryptosporidium spp, Giardia lamblia, Entamoeba histolytica, Ascaris lumbricoides,* hookworm infection, *Schistosoma spp* and *Strongyloides stercoralis* are important cosmopolitan intestinal parasites that are common among children and HIV/AIDS individual [6]. Clinical manifestation among children habouring these parasites include abdominal pain, nausea, reduced appetite, iron-deficiency anaemia, retarded growth and impaired cognitive performance [7,8].

2. METHODS

Makurdi has 104 meters elevation above the sea level. Makurdi lies within 8°30'E, 8°35 E and 7°30'N, 7°43'N. It has estimated population of 428913 with growth rate of 3% [9].

2.1 Study Population

2.1.1 Sample size

Sample size was determined using this formula:

$$S= X^2 NP (1-P) \div d^2 (N-1) + X^2P (1-P)$$

Where:

S = Sample size being sought
X^2 = Table value for chi-square at 1 degree of freedom at the desired alpha level (0.05 = 3.84: 01 =6.64)
N = Population size
P = The population proportion (usually 0.05 as this provides the maximum sample size).
D = Degree of accuracy desired, expressed as a proportion (usually 0.05).

A total number of 400 samples were collected and analyzed. The samples were collected from Bishop Murray Hospital Makurdi and 45 NAF Base Hospital Makurdi, these two Hospital run HIV clinical services weekly and also tend to have the highest number of HIV patients attending clinical service. A total number of 200 samples were collected from Bishop Murray Hospital Makurdi while 200 from 45 NAF Base Hospital Makurdi. Approval was gotten from the Hospital Management Board.

2.2 Method of Sample Selection

Table of random numbers was used to randomly select participant for this investigation at the selected Hospitals in Makurdi metropolis.

2.3 Collection of Fecal Samples

Each enrolled HIV/AIDS patient was asked to provide a fresh fecal sample in cleaned and dried sterile specimen bottles that were provided. The selected individuals were adequately instructed on how to get a little portion of their stool into the bottles using cardboard paper and applicator stick. Each participant was interviewed for sociodemographic variables [10]

2.4 Laboratory Procedure for Intestinal Parasite [11]

Each stool specimen was assessed for consistency. Then, it was examined by direct wet

mount method using normal saline (0.85% NaCl solution) in order to prevent the loss of motile stage of parasites [11]. Lugol's iodine was used to detect the cyst of intestinal parasites, also formal ether concentration techniques was used for the diagnosis. Faeces were emulsified by stirring 1 g of stool suspension in 10 ml of normal saline solution. The suspension was filtered into a centrifuge tube to remove large faecal particle before adding ether. The filtrate was centrifuged at 1,500 revolutions per minute for 3 minutes and the supernatant was discarded before keeping the tube in an upright position to allow water from the side to drain to the bottom. One to two (1-2) drops of sediment was transferred to a clean grease-free slide and then covered with a cover-slip and the preparation was examined microscopically using ×10 objective lens. The laboratory investigation was carried out at City Hospital Old G.R.A Makurdi. Diagnosis was based on the identification of helminth ova and protozoan cyst in the sample during microscopic examination. A person was considered to have a multiple infection if they were found to be positive for more than one species [10].

2.5 Collection of Blood Samples

Each enrolled person living with HIV/AIDS blood was collected. 3-4 ml of blood was collected. The selected individuals hand was inspected before deciding on a puncture site, then cleaned with methylated spirit, within the selected region, tourniquet was applied to increase the blood pressure, then 5 ml new syringe and needle was used to collect the blood through the vein and transfer into a plastic blood tube containing anti-coagulant (EDTA) and each participant was interviewed for sociodemographic variables.

Blood examination: Blood examination was done by thick films. These were examined with a light microscope, using oil immersion. Two to three (2-3) drops of blood were dropped on a clean microscopic slide and spread in a circular motion over a 2 cm area with the corner of another slide. The films were dried at room temperature with the slide position on a flat surface. The films were stain using Giemsa and allow to air dry for 30-45 minutes and then wash off using Physiological Buffer Saline (PBS). The blood film was allowed to dry at room temperature, then viewed using × 10 power objective to identify the parasites.

2.6 Packed Cell Volume (pcv)

The heparinized capillary tube was filled about three quarter of its length with blood by capillary movement; one end of the tube was sealed using plasticine sealant. The sealed capillary tube was placed on microhaematocrit centrifuge, with the seal end against the rim gasket and centrifuged for 3-5 minutes at 3000rpm. After centrifuging, three regions were form, the plasma, red blood cell and buffy coat. The packed red cell was read using a microhaematocrit reader and the result was interpreted in percentage (%).

2.7 White Blood Cell Count (wbc)

0.38 ml of Turk's solution was measured and dispensed into a tube, 0.02 ml of well mixed anti-coagulated blood (EDTA) was added and mixed and left undisturbed for 2 minutes. The chamber was charged by moistening each surface or each size of grid areas and a cover slip was pressed on each side until the Rainbow colour (newton ring) were seen. The diluted sample was remixed and 0.02 ml was pipette using disposable pasteur pipette which was used to fill or load one of the grid of the chamber and examined microscopically using ×10 objective lens.

2.8 CD4 Count

The values of CD4 were obtained through secondary data from the Hospitals.

2.9 Data Analysis

Ch-square test was use to test the association between intestinal helminthes and malaria infection amongst HIV patients at P=0.05. $\frac{\sum(0-E)2}{E}$

3. RESULTS

Table 1 the distribution of intestinal and Malarial Parasites by PCV level. The highest infection of (56%) was recorded among those who PCV is <18% while the least infection was recorded among those who have normal PCV with (3%). Chi-square test reveals that there is significant difference in PCV level (χ^2 Cal= 111.407 df 3, P<0.05).

Table 2 shows the distribution of intestinal and Malarial Parasites by WBC count level. 61% infection was recorded among those whose WBC

is extremely low while the least infection of (4%) was recorded among those that have normal WBC. Chi-square reveals that there is significant difference in level of WBC (χ^2 Cal= 121.662df 3, P<0.05).

Table 3 the distribution of intestinal parasites and *Plasmodium falciparum* by CD4 level. The highest infection of (56%) was recorded among those whose CD4 cells are extremely low, while the least infection was recorded among those whose CD4 cells is normal (9%). The Chi-square test reveals that there is significant difference in CD4 cell among people living with AIDS. (χ^2 Cal= 175.311 df 2, P<0.05).

4. DISCUSSION

The distribution of intestinal parasites and Malaria Parasites in relation to Packed Cell Volume (PCV) level according to World Health Organization standard, (34%) of Malaria Parasites was recorded among severe anemia (<18% PCV) and least infection of (2%) was recorded among those that have normal PVC, also 4% Hookworm was recorded among severe anemia while the least infection of (0%)was recorded among mild anemia and normal PCV; this result agrees with [1] who reported that intestinal and Malaria Parasites infections play an important role in the progression of HIV

Table 1. Distribution of intestinal and malarial parasites in relation to pack cell volume according to world health organization standard

Parasites	Severe anemia (<18%) A (%)	Moderate anemia (18-25%) A (%)	Mild anemia (26-30%) A (%)	Normal (31%^) A (%)	Total % A (%)	P. value
Malaria parasite	136(34)	30(8)	15(15)	5(2)	185(47)	0.000
Hookworm	15(4)	1(0.3)	0(0)	0(0)	16(4)	0.000
Fasciola spp	4(1)	1(0.3)	0(0)	0(0)	5(1)	0.180
Taenia solium	31(8)	14(4)	3(1)	2(1)	50(13)	0.000
Taenia seginata	9(2)	5(1)	2(1)	1(0.3)	17(4)	0.028
Entamoeba histolytica	10(3)	12(3)	7(2)	1(0.3)	30(8)	0.027
Hymenolepsis nana	6(2)	2(1)	1(0.3)	1(0.3)	10(3)	0.079
Giardia lamblia	2(1)	4(1)	0(0)	0(0)	6(2)	0.414
Ascaris lumbricoides	1(0.3)	0(0)	0(0)	0(0)	1(0.3)	0.049
Schistosoma mansoni	5(1)	3(1)	1(0.3)	0(0)	9(2)	0.264
Strongyloides stercoralis	1(0.3)	4(1)	0(0)	0(0)	5(1)	0.180
Total (%)	220(55)	76(19)	29(7)	10(3)	334(84)	

Table 2. Distribution of intestinal and malarial parasites by White Blood Cell (WBC) count level among people living with HIV/AIDS within Makurdi Metropolis

Parasites	Extremely low (<2000x10^9/L) A (%)	Moderate (2000-3900x10^9/L) A (%)	Normal (4000-11000x10^9/L) A (%)	Above normal (11000^) A (%)	Total % A (%)	P. value
Malaria parasite	167(42)	30(8)	2(1)	6(2)	185(46)	0.000
Hookworm	7(2)	7(2)	0(0)	0(0)	16(4)	0.210
Fasciola spp	2(1)	3(1)	0(0)	0(0)	5(1)	0.658
Taenia solium	21(5)	12(3)	9(2)	2(1)	50(13)	0.038
Taenia seginata	8(2)	4(1)	2(1)	1(0.3)	17(4)	0.181
Entamoeba histolytica	18(5)	7(2)	0(0)	1(0.3)	30(8)	0.007
Hymenolepsis nana	7(2)	3(1)	0(0)	1(0.3)	10(3)	0.206
Giardia lamblia	3(1)	2(1)	1(0.3)	0(0)	6(2)	0.607
Ascaris lumbricoides	1(0.3)	0(0)	0(0)	0(0)	1(0.3)	0.049
Schistosoma mansoni	5(1)	2(1)	0(0)	0(0)	9(2)	0.368
Strongyloides stercoralis	3(1)	0(0)	0(0)	0(0)	5(1)	0.655
Total (%)	241(60)	51(13)	14(4)	28(7)	334(84)	

Table 3. Distribution of intestinal and malarial parasites in relation to CD4 cells among people living with HIV/AIDS in within Makurdi metropolis

Parasites	Extremely low (<200Cell/µl) A(%)	Moderate (200-340Cell/µl) A(%)	Normal (350^Cell/µl) A(%)	Total % A(%)	P. value
Malaria parasite	132(47)	37(2)	16(11)	185(46)	0.000
Hookworm	9(2)	4(1)	3(1)	16(4)	0.144
Fasciola spp	5(1)	0(0)	0(0)	5(1)	0.206
Taenia solium	18(5)	21(5)	11(3)	50(13)	0.080
Taenia seginata	10(3)	4(1)	3(1)	17(4)	0.000
Entamoeba histolytica	24(6)	2(1)	4(1)	30(8)	0.206
Hymenolepsis nana	7(2)	3(1)	0(0)	10(3)	0.414
Giardia lamblia	4(1)	2(1)	0(0)	6(2)	0.020
Ascaris lumbricoides	0(0)	1(0.3)	0(0)	1(0.3)	0.047
Schistosoma mansoni	8(2)	1(0.3)	0(0)	9(2)	0.655
Strongyloides stercoralis	2(1)	3(1)	0(0)	5(1)	0.004
Total (%)	219(55)	78(20)	37(9)	334(84)	

infection, by further disturbing the immune system while it is already engaged in the fight against HIV.

The clinical effects of intestinal and Malaria Parasites are so numerous, in that the highest prevalence recorded cross the entire parasite are on those who are severely anemic (the Pack Cell Volume <18%), therefore the infect of intestinal and Malaria Parasites on red blood cells are so obvious, This result agrees with [12] who reported that Patients with some type of immune compromised condition and those submitted to immunosuppressive therapy whose Pack Cell Volume is low have an increased probability of acquiring parasitic infections, generally with a high degree of severity.

The distribution of intestinal and malaria parasites by White Blood Cell (WBC) count level among people living with HIV/AIDS within Makurdi Metropolis. The highest prevalence was *Plasmodium falciparum* with (42%), among those that have extremely low (<2000x10^9/L) White Blood Cells, while the least infection is recorded among those who have normal White Blood Cells (4000-11000x10^9/L) across the entire parasites. The result of this study contrast with the work of [13] Gastrointestinal problems resulting from opportunistic parasitic infections in HIV and AIDS (low white blood cells) infected subjects often present as diarrhoea and significant disease has been recorded in 50-96% of cases worldwide with 90% prevalence rate reported in Africa.

The clinical effects of intestinal and malaria parasites on individuals whose white blood cells are low (extremely low <2000x10^9/L and moderate 2000-3900x10^9/L) are obvious, in that the highest prevalence recorded cross the entire parasite are on those whose white blood cells are extremely low and moderate, this imply that the level of white blood cells is a significant factor in the infection of parasitic disease among people living with HIV/AIDS. The result agrees with [14], the immune response of an immunocompetent host against parasites is a complex system in which both cellular and humoral defence mechanisms intervene. The result further agrees with [1] who reported that intestinal parasitic infections play an important role in the progression of HIV infection, by further disturbing the immune system while it is already engaged in the fight against HIV.

The increase in the prevalence of parasite disease among individuals whose White blood cells is low could be attributed to inability of the cell to produce needed antibody due to lack of sufficient white blood cells in the body; this result agrees with [1] who reported that intestinal parasitic infections play an important role in the progression of HIV infection, by further disturbing the immune system while it is already engaged in the fight against HIV. The distribution of intestinal and Malaria Parasites in relation to CD4 cells among people living with HIV/AIDS within Makurdi Metropolis, The highest prevalence of Malaria Parasites recorded is (47%) among those that have extremely low (<200Cell/µl) CD4 cells, while the least infection is recorded among those who have normal CD4 Cells (350^Cell/µl) across the entire parasites; This work agrees with Zheng [15] who reported that with the progressive development of AIDS, especially once $CD4^+$ T-lymphocyte counts have fallen

below 200 cells/µl, patients often become co-infected by bacteria, parasites, or viruses. Chi-square test reveals that there is significant difference in the distribution of Malaria Parasites and *Taenia seginata* across CD4 Cells on the people living with HIV/AIDS; this means that the CD4 Cells level is a significant factor in the infection of immunosuppressed HIV/AIDS by the affirmed parasites. This result agrees with [16] HIV infection leads to loss of $CD4^+$T cells, which leaves affected individuals mortally susceptible to opportunistic infections.

The CD4 is a defensive cell, therefore reduction in the CD4 level to <250Cell/µl could leady to easy access of opportunistic diseases to thrive at already immunosuppressed individuals there by making the individual susceptible to parasitic infection; this work agrees with previous report [6] HIV infection leads to loss of $CD4^+$T cells, which leaves affected individuals mortally susceptible to opportunistic infections, many of the opportunistic infections that ultimately plague such individuals involve infectious agents that are normally checked by the mucosal barriers which include *Cryptosporidium spp, Giardia lamblia, Entamoeba histolytica, Ascaris lumbricoides,* hookworm infection, *Schistosoma spp* and *Strongyloides stercoralis* are important cosmopolitant intestinal parasites that are common among children and immune-compromised individual.

The determine factor on the degree of infection of HIV/AIDS on individual is by the level of the CD4 count. This result agrees with Hunter et al. (2002) and Alakpa et al. [3,4] who reported, pathogenic intestinal parasites such as *Cryptosporidium* sp*, Cyclospora* sp*, E. histolytica* and *Giardia* sp, can last for months in patients with AIDS, causing malabsorption of nutrients, gradual debilitation through dehydration, and metabolic abnormalities and are responsible for severe diarrhoea episodes.

Parasitic infections remain an important cause of morbidity and mortality in developing countries especially among HIV/AIDS infected persons. The low prevalence of intestinal and Malaria Parasites recorded among individual whose CD4 cell is normal (350^Cell/µl) could be attribute to abundant of a glycoprotein found on the surface of immune cells as T-helper cells, monocytes, macrophages, dendritic cells and white blood cells that are an essential part of the human immune system. This result agrees with Brady et al. [17] who reported that CD4 is a co-receptor that assists the T cell receptor (TCR) in communicating with an antigen presenting cells, using its intracellular domain; CD4 amplifies the signal generated by the TCR by recruiting an enzyme which is essential for activating many molecular components of the signaling cascade of an activated T cell.

5. CONCLUSION

In this study, the prevalence of intestinal and Malaria Parasites infection among the HIV/AIDS individual was high and there by posing danger or threats now and in near feature. From the result obtained in this research work one can conclude that all the HIV/AIDS persons whose CD4 cell, PCV and WBC cells were low has at least one infection or the other, ranging from either intestinal or Malaria Parasites or both, also school age children, pregnant women are highly susceptible to parasitic infection due to poor personal hygiene, improper disposer of human faces, entering endemic areas, and contact with contaminated soil. Therefore all HIV/AIDS persons are at high risk of parasitic infections.

CONSENT

Informed consent was obtained from each of the participants and or their parents/ guardians in case of children prior to specimen collection.

ETHICAL APPROVAL

Approval to carry out this study was granted by the Benue State Hospital Management Board after a letter of introduction and research proposal was presented to the Hospital Management Board.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DOI: 10. 1126/science.8493535
[PMID 8493535]

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/58260