Original Article

Serosurvey and factors associated with *Leishmania Donovani* infection in febrile HIV infected individuals attending Abuja Teaching Hospital, Nigeria

[1]I. N. Abdullahi, [2]H. A. Shuwa, [3]A. U. Emeribe, [4]L. O. Olayemi, [5]T. Yunusa, [6]A. E. Ahmad, [7]P. O. Musa

[1]Department of Medical Laboratory Science, College of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Community Health, Federal University, Dutse, Nigeria
[3]Department of Medical Laboratory Science, University of Calabar, Calabar, Nigeria
[4]Department of Medicine, National University of Samoa, South Pacific
[5]School of Medical Microbiology and Parasitology, University of Abuja, Abuja, Nigeria
[6]Department of Medical Laboratory Science, College of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria
[7]Department of Medicine, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT

Background: *Leishmania Donovani* (L. donovani) is an obligate intracellular pathogen. *L. donovani* and HIV co-infection is neglected clinical entity in sub-Saharan African. *L. donovani* infections have been shown to deplete host cellular immunity and proceed to severe diseases. As part of the ongoing research efforts in Nigeria to improve the healthcare of HIV infected individuals through diagnosis and treatment of co-infections with leishmaniasis, this study was instigated.

Methods & Materials: Three hundred and forty blood samples were individually collected from HIV infected individuals with fever>10 days attending University of Abuja Teaching Hospital, Nigeria. EDTA anticoagulated blood was tested for CD4+ cell counts, while sera were tested for *L. donovanii* IgG antibodies using flow cytometry and Enzyme linked immunosorbent Assay (ELISA) respectively. Interviewer-based questionnaires were used to collect participants' sociodemographic variables. Data were analysed for statistical association and relation between seropositivity of *L. donovani* IgG and risk factors.

Results: Of the 340 participants studied, the seroprevalence of *L. donovanii* antibodies was 8.2%. The mean $CD4^+$ cell count of those with *L. donovani* seropositivity (n = 28) was 119.4cells/mm^3. There was statistical relation between CD4+ cell counts and L. donovani antibodies. There was statistical association between *L. donovani* IgG seropositivity with age of participants (p = 0.014), residential area (p = 0.033), living condition (p = 0.0006) and **Proximity to water collection** (p<0.0006) and **bushes/ vegetation (p=0.049)**. Skin disfiguration was significantly associated with *L. donovani* IgG antibodies (p=0.000).

Conclusion: Findings from this study revealed that *L. donovani* is an etiological agent of acute fever and skin disfiguration in HIV infected patients and significantly associated with CD4+ lymphopaenia in HIV co-infectedpatients.

Corresponding Author
Idris Nasir ABDULLAHI,
Office B7, Department of Medical Laboratory Science,
College of Medical Sciences,
Ahmadu Bello University, Zaria, Nigeria
E-mail: eedris888@yahoo.com
Tel.: +2348022851352

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INTRODUCTION

Leishmania donovani (L. donovani) is an obligate intracellular pathogen. *L. donovani* and human immunodeficiency virus (HIV) co-infection is neglected clinical entity in sub-Saharan African. Leishmaniasis – HIV co-infection can present itself as cutaneous, mucocutaneous or visceral leishmaniasis[1]. Usual and unusual manifestations of cutaneous/mucocutaneous leishmaniasis have been reported mainly in Africa and South America, but the real prevalence of *Leishmania donovanii* infection in HIV-infected patients is not clear[1].

The World Health Organization reported that about 700,000-1,000,000 incidence of leishmaniasis with 20 000 to 30 000 deaths occur annually. Out of these, approximately 500,000 are of visceral leishmaniasis and 800,000 cutaneous/mucocutaneous leishmaniasis[2]. Leishmaniasis has been endemic in over 98 countries and territories[2]. It affects mainly some of the poorest people on earth, and is associated with malnutrition, population displacement, poor housing, immunosupression, and poor financial resources [2].

The spread of leishmaniasis is linked to environmental changes due to deforestation, building of dams, irrigation schemes, and urbanization [2]. HIV infection is a major global health problem that affects about 36.9 million people living with HIV and over 2.0 million new infections annually [3]. HIV is present in practically all parts of the world territories; however, the major burden of the disease is in sub-Saharan Africa [4]. Over the years, an overlap between the transmission areas of HIV and leishmaniasis has been observed. As a result, there have been an increasing number of cases of HIV–*Leishmania* co-infection, which has spread throughout the world. Since the last four decades, *Leishmania*–HIV co-infection has been reported in 35 countries [1].

Although mucocutaneous leishmaniasis develops in only a small number of patients with New World cutaneous leishmaniasis, its course is chronic and may be life-threatening[5]. The physical effects of Leishmaniasis can range from mild scarring to gross disfiguration and death, so its presence in a community is often very obvious and disturbing[5]. *Leishmania* and HIV each promote the activation of the other, causing host immune impairment. *L. donovani* infections have been shown to deplete host cellular immunity and proceed to severe diseases, antiretroviral therapy (ART) treatment, high relapse, and high mortality rate [1,5]. In most of the studies done outside Nigeria, factors associated with HIV-Leishmania co-infection are advanced HIV-associated diseases, intravenous drug users, CDC clinical category C and CD4 cell count below 300 cells/mm[3,6,7,8]. Nevertheless, one hospital based case series study done in Ethiopia showed that age was significantly associated with HIV-Leishmania co-infection [9]. Therefore, there is a scarcity of data on factors associated with HIV/Leishmaniasis co-infected patients in Nigerian settings.

Leishmaniasis diagnosis relies on clinical methods, but serological tests are used to diagnose leishmaniasis despite them having a low sensitivity to cutaneous/mucocutaenous leishmaniasis[11]. The search for the parasite is used to diagnose both visceral leishmaniasis and cutaneous /mucocutaneous leishmaniasis. However, Enzyme linked immunosorbent assay (ELISA) is one of the standard methods to detect leishmaniasis[10]. Antibodies occurpredominantly in visceral leishmaniasis and their detection can confirm a diagnosis. Several testing methods have been employed in the diagnosis of acute visceral leishmaniasis[10]. For example, indirect immunofluorescent antibody tests (IFAT) and direct agglutination tests (DAT) are two sero-diagnostic procedures in practice because the anti-leishmanial antibody titers are generally high at the acute stages[11].

One method that is less successful is the aspiration of bone marrow. Besides being a painful and risky procedure, its success rate is not very high. Alternatively, the ELISA method is the favoured

sero-diagnostic procedure, which is sometimes used in conjunction with the Immunofluorescence antibody and direct agglutination tests[10]. Drugs available to treat leishmaniasis are more restricted and cause severe side effects[11]. Furthermore, in HIV-infected patients, these side effects are more prominent and relapses and lethality are more recurrent[11].

Despite the high HIV burden in Nigeria, there is paucity of information of the true prevalence of *Leishmania* infection among HIV infected persons in Abuja Suburb. In view of this, the present study aimed to determine the prevalence and risk factors associated with *Leishmania donovani* infection among HIV infected persons in Abuja, North-central Nigeria.

MATERIALS AND METHODS

Study design

This was a cross-sectional study conducted using blood samples from 168 antiretroviral-therapy (ART) naïve and 172 ART experienced HIV-infected individuals. All participants aged between 15 and 50 years were screened and their HIV status was confirmed using Uni-Gold Recombigen® HIV-1/2 (Trinity Biotech, Wicklow, Ireland) and Determine™ (Alere, Auckland *city*, New Zealand) proprietary reagents. These samples were collected at the HIV Clinic of the University of Abuja Teaching Hospital (UATH), Gwagwalada, Abuja, Nigeria.

Study area

This hospital-based research was conducted at UATH in the federal capital territory, Abuja, Nigeria. New cases of HIV infections are diagnosed in this tertiary hospital, and those undergoing therapy are also monitored. Blood samples were collected at the PEPFER Special HIV clinic and analyzed at the immunology laboratory.

Informed consent and ethical approval

The study was explained to the enrolled participants, and they gave their written informed consent. Parents/guardians gave approval for the participants who were children. Participants were all confirmed seropositive for HIV. An interviewer-based questionnaire was used to obtain bio-data and risk factors variables from these participants in accordance with the Declaration of Helsinki. Parents/guardians filled questionnaires on behalf of their children. Those who were sero-negative for HIV, had not given consent, or had other etiologies with similar pathologies, including active tuberculosis and non-tuberculosis mycobacterial infection, as well as other bacterial infections were excluded from this study. Ethical approval was obtained from the Ethical Research Committee of the UATH, Abuja. Data generated were anonymously analyzed throughout the study.

Sample size calculation

The sample size for this study was derived using data from a cross-sectional study conducted in Jos, Nigeria, by Igbe *et al*[12]. Thus, the minimum sample size required for this study was 40 using a 5% error margin and 95% confidence interval. However, statistical credence was given to this study by increasing the sample size to 340. Therefore, a total of 340 volunteers were recruited as participants for this study.

Sample collection and preparation

Whole blood samples of 2mL were collected aseptically in ethylenediaminetetraacetic acid containers and used for CD4+ cell counts and 2mL in plain container were obtained from the participants for serology. Samples were collected between from 7th April to 10th October 2015. Blood were analyzed within 1 h of collection.

Laboratory analytical procedures

Determination of cluster of differentiation($CD4^+$) cell count

Based on the manufacturer's instructions, the $CD4^+$ cell counts in the whole blood were analyzed using a Partec™ CyFlow Analyzer (Sysmex, Norderstedt, Germany) Model SL3. This device used the

principle of light scattering property (based on dissimilarity in cell size or granularity) and the fluorescence of cells following staining with monoclonal antibodies to markers on the cell surface bound to fluorescent dyes. Cell populations of interest were then gated after identification. Absolute $CD4^+$ cell counts were subsequently analyzed using a single-platform technique.

Determination of Anti-Leishmania donovani IgGantibody

ELISA was carried out according to the method described by kit manufacturer (Abnova ®). The *Leishmania donovani* ELISA test is a three-incubation process whereby the first incubation involved the coating of the wells with *Leishmania donovani* antigen. During this step, any antibodies that are reactive with the *L. donovani* antigens, bind to the wells. Next, the wells were washed to remove test sample. At this point Enzyme Conjugate was added. During this second incubation, the Enzyme Conjugate bound to any antibodies present. Before the third incubation step, 3 washings were done. Then a chromogen (tetramethylbenzidine) was added. With the presence of Enzyme Conjugate and the peroxidase causing the consumption of peroxide, the chromogen changed to a blue color. The blue color turned to a bright yellow color after the addition of the stop solution, which ends the reaction. ELISA readers were used to obtain the optical density of the final colored product. Subsequently, the results were calculated.

Statistical analysis

Data obtained were analyzed using SPSS software version 24 (IBM Corporation, Armonk, NY, USA) and were presented as percentages and mean ± standard deviation (SD). Student's *t*-test was used to compare continuous variables, while the Chi-square was used to compare categorical variables. A $p \leq 0.05$ at a confidence interval of 95% was considered statistically significant.

RESULTS

Socio-dermographic Characteristics

A total of 340 HIV sero-positive participants were enrolled in this study. Out of these, 158 (46%) were males, while 183 (54%) were females. Majority 142 (42%) of the study participants were in the age group of 25-34 years old. Among the study groups, 182 (44%) were illiterate and 152 (45%) were married followed by 104 (31%) single participants. The majority of study participants 182 (54%) were from rural residents (table 1).

Leishmaniasis-HIV Co-infection, Socio-dermographic Characteristics and Clinical Aspects of Leishmaniasis-HIV case-participants

Out of the total HIV suspected cases, 3 (25%) Leishmaniasis-HIV co-infection was found in the age groups of >44 years 3 (25.0%) followed by 15 (10.6%) within 25-34 years old. Leishmaniasis-HIV co-infection by gender showed that 19 (10.4%) of females and 9 (5.7) of males were co-infected by Leishmaniasis-HIV.Individuals who live in the rural 17 (19.3%), suburb 4 (5.9%) and urban 7 (7.8%) were co-infected with Leishmaniasis-HIV. Based on educational status, Leishmaniasis-HIV co-infections were found in 13 (13.5%) of participants with primary level of education followed by 6 (9.3%) of participants with secondary level of education. Leishmaniasis-HIV co-infection by marital status revealed that 6 (24.0%) of divorced participants followed by 9 (15.3%) of widows and widowers were co-infected by Leishmaniasis-HIV. This indicated statistically significant association between Leishmaniasis-HIV co-infection and marital status (p=0.001) (table 1). The proportion of participants with skin disfiguration and Leishmaniasis-HIV co-infection were significantly lower compared to those with skin disfiguration and HIV mono-infection ($x^2 = 47.2$; $p = 0.000$) (figure 2).

Leishmaniasis-HIV Co-infection and Immune Status

The mean (standard deviation) $CD4^+$ T-cell counts for participants both with Leishmaniasis-HIV co-

infection and HIV mono-infection were 119.4±26 cells/mm^3 and 231.7±76 cells/mm^3 respectively. The CD4$^+$ T-cell counts of participants Leishmaniasis-HIV co-infection were significantly ($p<0.000$) lower compared to those with HIV mono-infection (table 2).

Prevalence of Leishmaniasis-HIV Co-infection

Out of the total HIV participants, the overall prevalence of Leishmaniasis-HIV co-infection was 28 (8.2%). The prevalence of HIV mono-infection was 312 (91.8%) (Figure 1).

Associated Factors for Leishmaniasis-HIV Co-infection

From the total study participants, 124 (36%) used insecticide sprays. Of these, 6 (4.8%) were Leishmaniasis-HIV co-infected. Most of the study participants, 177 (52%) resided in areas located over 50 meters from bushes and vegetation. Of these, 5 (2.8%) were Leishmaniasis-HIV co-infected which was significantly ($p=0.049$) lower compared to their counterparts who reside less than 50 meters form bushes and vegetation. Out of the 89 (26%) who resided close to water collection sites, 15 (16.9%) were Leishmaniasis-HIV co-infected. Of the 172 (51%) of participants on anti-retroviral therapy, 10 (5.8%) were co-infected with Leishmaniasis-HIV, which was lower compared to those that are not on therapy although not significant ($p=0.134$).

Table 1: Distribution of Leishmania donovani IgG antibody by Sociodemographic characteristics of HIV infected persons

Variables	Observation	No. tested	No. anti-Leishmania positive (%)	*p-value*
Age, years (mean ±SD)	31.2 ± 12.9	340	NA	
Gender	a. Male	158	9 (5.7)	
	b. Female	182	19 (10.4)	
	Total	340	28 (8.2)	0.112
Age range (years)	a. <15	24	1 (4.2)	
	b. 15 -24	87	4 (4.6)	
	c. 25 – 34	142	15 (10.6)	
	d. 35 – 44	75	5 (6.7)	
	e. > 44	12	3 (25.0)	
	Total	340	28 (8.2)	0.100
Place of residence	a. Village/rural	182	17 (9.3)	
	b. Suburb	68	4 (5.9)	
	c. Urban	90	7 (7.8)	
	Total	340	28 (8.2)	0.664
Educational level	a. No formal education.	148	7 (4.7)	
	b. Primary	96	13 (13.5)	
	c. High school/Secondary	64	6 (9.3)	
	d. College	28	2 (7.1)	
	Total	340	28 (8.2)	0.108
Marital Status	a. Single	104	3 (2.9)	
	b. Married	152	11 (7.2)	
	c. Divorced	25	6 (24.0)	
	d. Widow/widower	59	9 (15.3)	
	Total	340	28 (8.2)	0.001*

Table 2: Comparison of CD4+ T-cell count of HIV-infected participants with and without *Leishmania donovani* IgG antibodies enrolled for the study.

Group	$CD4^+$ T-cell counts (cells/mm^3)	
	Range	
Participants HIV and anti-L donovani IgG + (n =28)		119.4±26
Participants HIV and anti-L donovani IgG - (n =318)	188 – 916	231.7±76
t-statistic value	-	7.769
Df		7.769
p-value	-	<0.0001*

*Significance determined by student t-test

Table 3: Risk factors of *Leishnamia donovani* IgG antibodies in HIV infected persons

Variables	Observation	No. Tested	No. anti-Leishmania positive (%)	*p-value*
Income per month (US $)	a. < 50	232	16 (6.9)	
	b. 50 - 200	45	5 (11.1)	
	c. >200	63	7 (11.1)	
	Total	340	28 (8.2)	0.421
Occupation	a. Farmer	124	9 (7.3)	
	b. Civil servant	36	4 (11.1)	
	c. Driver	19	1 (5.3)	
	d. Student	67	7 (10.4)	
	e. Unemployed	94	7 (7.4)	
	Total	340	28 (8.2)	0.864
Frequent use of residual insecticide spray	a. Yes	124	6 (4.8)	
	b. No	216	22 (10.1)	
	Total	340	28 (8.2)	0.084
Proximity to bushes/ vegetation	a. < 50 meters	92	12 (13.0)	
	b. > 50 meters	248	16 (2.8)	
	Total	340	28 (8.2)	0.049*
Living condition	a. Sleep on the floor	54	5 (9.3)	
	b. Good living condition	107	2 (1.9)	
	c. Mud houses	62	9 (14.5)	
	d. Over-crowded in rooms	117	12 (10.3)	
	Total	340	28 (8.2)	0.021*
Proximity to water collection	a. Yes	89	15 (16.9)	
	b. No	251	13 (5.2)	
	Total	340	28 (8.2)	0.0006*
On Antiretroviral therapy	a. Yes	172	10 (5.8)	
	b. No	168	18 (10.7)	
	Total	340	28 (8.2)	0.134

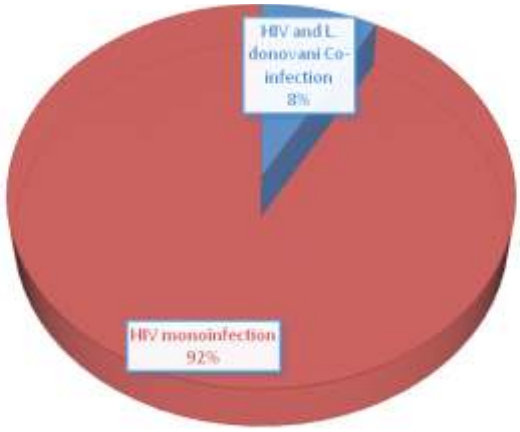
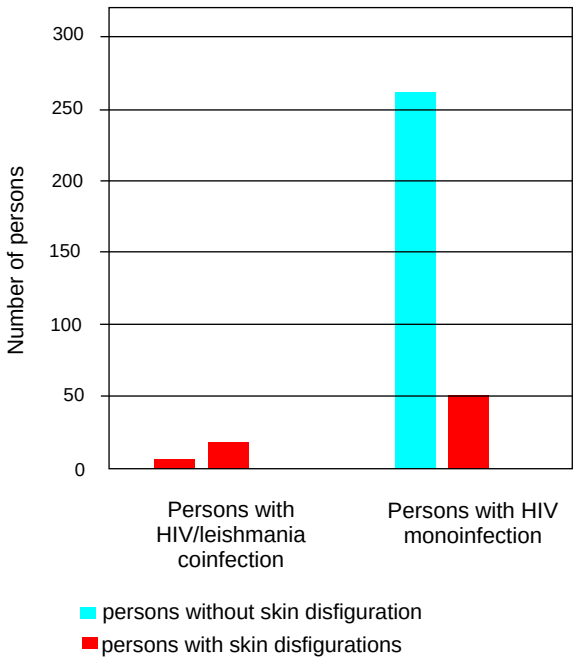


Figure 1: Seroprevalence of anti-L. donovani IgG among HIV infected persons



Df = 1; X^2 =47.2; p value = 0.000

Figure 2: Comparison of *L. donovanii* IgG antibody with skin and without disfiguration in HIV infected persons.

DISCUSSION

Leishmaniasis/HIV coinfection is considered an emerging problem in several countries despite the growing number of cases, a problem of late diagnosis occurs thereby, worsening the prognosis. There has been an observed overlap in the transmission pattern of Leishmaniasis and HIV [1], but their co-infection has been poorly investigated with paucity of reports especially within the West African region. In this present study, the overall prevalence of Leishmaniasis- HIV co-infection was 8.2%. This is in agreement with the study conducted in Brazil, which reported the incidence of this co-infection at 8.5% in 2014[13]. However, this prevalence was lower as compared to previous studies conducted in Ethiopia with reported co-infection prevalence between 23-49% [9,14 - 19] and higher than a study conducted in India with prevalence rate of 2.18% [11]. This variation may be due to the level of awareness of Leishmaniasis, and environmental factors including deforestation, dam construction, irrigation schemes, and urbanization, which the disease spread has been associated with. On the other hand, Ethiopia serves as one East African Countries besides South Sudan and Sudan with over 90% of global Leishmaniasis cases [20].

Regarding marital status-related prevalence of anti-Leishmaniasis IgG from this study, married participants had higher prevalence compared to unmarried participants, but a lower prevalence was observed compared to those separated/divorced and bereaved. This finding was in line with those of Uranw *et al* [18] who reported a strong association between marital status and the local transmission of Leishmaniasis (p=0.02).

Findings from this study revealed a higher prevalence of Leishmaniasis IgG seropositivity in the study participant who were exposed to environmental risk factors including proximity of less 50 meters to bushes/vegetation and poor living condition (e.g. residing in mud/thatched houses, sleeping on the floor, overcrowded accommodation) compared to those not exposed to these risk factors. These findings were in accordance to the previous study conducted in Eastern Nepal [18], which revealed that sleeping on the floor (p=0.000), residing in thatched houses without windows (p=0.001), regular forest visits (p=0.000), large households of more

than 5 persons (p=0.001) among other factors were strongly associated with the local transmission of Leishmaniasis. Although, occupation and outdoor activity was also considered as a potential risk factor for Leishmaniasis, there was no strong association observed between occupation and the disease. This is in agreement with the study conducted in an endemic region of Sri Lanka[21], which reported similar observation and suggested that the absence of an association between the disease condition and occupation could be due to the existence of potential reservoir hosts in the peridomestic environment (sylvatic cycle).

The mean CD4+ T lymphocytes observed in this study for HIV-Leishmania co-infected patients was 119.4 cells/ mm3 while in those with HIV mono infection it was 231cells/mm^3. However, statistical credence is given to the fact that HIV- Leishmania co-infected patients had a much lower CD4+ T cell count(p= 0.001) compared to those with HIV monoinfection. The reason for this might be ascribed to the immune impairment characteristic of *L. donovani* which is commonly seen in HIV co-infected patients.

This finding is often times associated with a greater likelihood of relapse in this group of patients due to pronounce lymphopenia [22]. Moreso, the low CD4+ T cell counts seen in this study, quite acquiesces with a study in Brazil which reported low CD4+ T lymphocytes of 51cells/mm^3 in a patient with marked desquamation and ulcerated lesions with up to 90% of cases having CD4+ T cells less than 200 cells/mm^3. This is imperative in situation of reactivation or relapse and putting into consideration the need for timely initiation of highly active antiretroviral therapy in co-infected patients. Findings from this study may however, suggest a possibility of misdiagnosis as HIV-Leishmania co-infected patients express longer and intermittent duration of symptoms, thus an inability of the health care worker to include kala azar in the differential diagnosis while investigating other opportunistic pathogens that have similar clinical presentations e.g Mycobacteriosis or disseminated histoplasmosis.

Findings from this study revealed a strong association of L. donovani infection with skin disfiguration both in HIV-leishmania co-infected and HIV mono-infected patients (DF=1, p<0.000). The reason is attributed to immunosuppression in HIV infected patients and transplantation cases as being reported in a number of Cutaneous and mucocutaneous leishmaniasis [24].

CONCLUSION

The findings from this study revealed that *L. Donovani* is an etiological agent of acute fever and skin disfiguration in HIV infected patients and thus, significantly associated with CD4+ lymphopaenia in HIV co-infected patients. However, the control of Leishmaniasis requires a combined effort at vector control, improved living conditions, case detection, early treatment and appropriate management. Early detection, diagnosis and treatment are crucial for individual patients and for the community. Untreated Leishmaniasis patients serve as reservoirs for parasites and therefore provide disease transmission in high risk environments.

CONFLICT OF INTEREST: None

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