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Updates on Malaria parasites distribution among HIV infected and AIDS patients in Comboni Hospital, Rubirizi District, Uganda

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Abstract:
Background: Despite efforts to clamp down the pandemic of HIV/AIDS, its impact is still alarming. Epidemics are increasing due to man-made conflicts and climate-associated disasters causing the movement of non-immune populations to malaria-endemic areas. Urban and peri-urban malaria is now a substantial problem in Africa with Malaria becoming more difficult to manage.
Objectives: To outline the pattern of malaria parasites distribution among HIV/AIDS patients in Comboni Hospital, Uganda.
Methods: This was a cross-sectional, descriptive study, designed to investigate the malaria prevalence in 160 febrile HIV/AIDS patients. Thick and thin blood films were made and stained with field stains A and B, guided by standard parasitological methods, and examined microscopically under x 100 oil immersion objective.
Results: A total of 160 HIV/AIDS patients were recruited between August and October 2013. The prevalence of Malaria in HIV was 30(19%) of the participants were co-infected with both Malaria and HIV while 130(81%) had only HIV. Most had CD4 cell count between 600-<800 at 79(49%) while those with CD4 cell count below 200 were only 10(5%). The prevalence of HIV and malaria was 33.3% high among working-class people of ages 30-40 years. This could be linked to their occupation which encourages them to work too late at night which exposes them to mosquito bites. Immunosuppression or CD4 level inversely impacted the malaria distribution. Thus low CD4 was associated with high malaria distribution and high malaria distribution was common with participants with low CD4 count. Other significant results are fully discussed.
Conclusion: HIV was high among Malaria patients and malaria was high among HIV-infected and AIDS patients. Mosquito net usage was effective in malaria control among those who used them but was ineffective among some age groups characterized by constant stay outside the mosquito net due to alcoholism and a variety of social events which makes people stay outside the nets at nights.

Introduction

Malaria is a vector-borne disease caused by *Plasmodium* species transmitted through the bite of female anopheles mosquitoes. It is a major public health problem and cause of much suffering and premature death in the poorer areas of tropical Africa, Asia, and Latin America. There are four species of *Plasmodium* species that cause human malaria; *Plasmodium falciparum*, *Plasmodium vivax*: *Plasmodium malariae,* and *Plasmodium ovale.* Plasmodium completes its life cycle in two phases: man the intermediate host and female anopheles mosquito which are the definitive host. The sporozoites are the stage of plasmodium that is injected into the human during a mosquito blood meal (1). Besides mosquito bites, infection to man can occur during blood transfusions and through trans-placental transmission. Infection of malaria is more prevalent in tropical and sub-tropical countries and is characterized by classical tertian and quatrain types of fever. Plasmodium falciparum is known to cause deadly complicated drug-resistant malaria. Diagnosis of malaria is by detecting the parasites in blood smears, serology, polymerase chain reaction, and use of rapid test kits. (1).

Malaria contributes to a temporary increase in viral load among HIV-infected people which may worsen clinical disease and increase mother-to-child transmission and transmission in adults. Malaria also causes anemia which often requires blood transfusions, a procedure that increases the risk factor for HIV infection where universal blood screening has not been achieved. The high prevalence of both HIV and malaria infection in Africa means that even small interactions between the two could have substantial effects on the populations (2). Although the effect of malaria on HIV has not been well documented, acute malaria infections are known to increases the viral load, leading to increased transmission of HIV and more rapid disease progression (3). It is estimated that 29.4 million Africans are infected with HIV1 whereas at least 500 million suffer from malaria each year. Therefore, any interaction between these two infections will be of major public health significance (2).

Despite efforts to clamp down the pandemic of HIV infections and AIDS in most developing countries, its impact is still alarming. Epidemics are increasing due to man-made conflicts and climate-associated disasters causing the movement of non-immune populations to malaria-endemic areas. Urban and peri-urban malaria is now a substantial problem in Africa with Malaria becoming more difficult to manage.

In recent times, malaria and HIV infection have drawn much research attention. Over 80% of malaria deaths in the world are known to occur in Africa, mostly in children under five years of age. Malaria and HIV frequently result in co-infection and interactions between the two diseases may therefore have major implications for the treatment, care, and prevention of both. However, patients in Comboni hospital attending HAART clinic are not routinely screened for malaria infection and therefore the prevalence of malaria in HIV is undocumented, thus the purpose of this research (4, 2). This study was designed to outline the pattern of malaria parasites distribution among HIV infected and AIDS patients in Comboni Hospital in Uganda with the ultimate goal of providing effective interventions.

Material and Methods

This study was conducted at Comboni Hospital Kyamuhunga, a private not-for-profit (PNFP) Hospital, located in Ryabagoma Village Kyamuhunga Sub-County, Igara West Constituency, Bushenyi District. It is approximately 80km from Mbarara town on Mbarara -Kasese High way and 15km from Bushenyi District headquarters. The main health problems are Malaria, Respiratory infections HIV/AIDS, and skin infection.

This was a cross-sectional, descriptive study designed to investigate the malaria prevalence in febrile HIV infected and AIDS patients. The 160 samples analyzed was obtained using the formula: $n=Z^2pq/d^2$, where n = sample size, (5), P = prevalence from records of malaria in HIV patients (in proportion of one; if 11.8% P = 0.118) (6); d = error (in proportion of one; if

5%, $d = 0.05$). Z = Z statistic for a level of confidence Z statistic (Z). The 160 people included were febrile patients between 10 years to 60 years of age and that are positive for HIV. HIV patients who had no malaria parasite served as control. CD4 count of healthy volunteers was used as the normal range values and besides patients, age, sex, and social activities were also obtained. Those excluded were infants/elderly, HIV-negative patients who were on antimalarial treatment.

A 5 ml blood sample was obtained by vena-puncture from each of these patients into EDTA anticoagulant bottles. A sample of blood was transferred from the EDTA bottle onto a test kit for malaria rapid test kit to test for *falciparum* malaria. Thick and thin films were prepared from each subject's blood sample**.** The thin films were fixed with absolute methanol and both thick and thin films were stained with fields stain A and B after which they were examined microscopically under oil immersion under x 100 objectives. The parasite counting was done using the thick blood films, while the thin blood films were used for confirmation of the malaria parasite species. The study was approved by appropriate bodies and informed consent was sought and obtained from the participants. Thus the HIV/AIDS patients participating in the study were volunteers with informed consent properly sought and obtained after due process.

Quality was maintained through aseptic sample collection, processing, and analysis using standard parasitology methods. Data collection and analysis were done using up-to-date statistical packages. The quality of samples used was only non-haemolysed blood samples. Positive and negative controls were used to test the functionality of each batch of malaria test strips before being used for malaria testing.

Results

In this study, a total of 160 HIV/AIDS patients were recruited between August to October 2013. The prevalence of Malaria in HIV shows that 30(19%) of the participants were co-infected with both Malaria in HIV while 130(81%) had only HIV. On the other hand, most had CD4 cell count between 600-<800 at 79(49%) while those with CD4 cell count below 200 were 10(5%).

Table 1 shows that of the 160 participants studied, only 30 (19%) of the malaria positive results were from participants with CD4 cell count below and none was found among patients with hat higher CD4 cell levels. This underscores the importance of host immunity in the distribution of malaria parasites especially among **populations living in resource-limited settings.**

Table 2 shows high levels of malaria at the age of 30-<40 at 10(33.35%) and low levels above 50 years at 14(6.5%). Both female and male participants had an equal prevalence of co-infection each at 15(50%). In marital status, single participants had more cases of malaria at 18(60%) while married participants were at 12(40%). Farmers accounted for the highest level of malaria with up to 12(40%). Of the participants 100 consumed alcohol with 19(63.3%) while those who did not consume alcohol were 60 accounting for 11(36.7%). Most participants used mosquito nets at 130 with only 4(10.3%) having been positive for malaria. 30 participants did not use a mosquito net with 26(89.7%) having malaria parasites. Most participants were educated with only 18 uneducated.

Table 3 shows the malaria status of female and male participants of various ages. It shows that the total number of participants with both HIV and malaria co-infection was 30(19%) with equal numbers of males and females (fifteen each). The age group with the high level of infection was 30-40 years with had 10(33.3%).

Table 4: Shows the demographic addresses of participants and their malaria status majority of the participants with both HIV and malaria were from Ndekye with 25(15.63%) and participants from other places had the least at 4(2.5%). Most of the participants were farmers with 53(33.13%) and the least participants were unemployed with 21(13%).

Table 1 CD4 cells count distribution among recruited participants

CD4cellcount * Malaria status			
CD4cellcount	**Negative**	**Positive**	**TOTAL**
<200	0(0.0)	5 (16%)	**5(3.14%)**
>1000	12(9.3)	0	**12(7.55%)**
200- <400	1(0.78%)	25(83.3%)	**26(16.4%)**
400- <600	44(34.11%)	0	**44(27.7%)**
600- < 800	49(37.98%)	0	**49(30.8%)**
800- <1000	24(17.8%)	0	**23(14.5%)**
TOTAL	**130(81.13 %)**	**30(19%)**	**160(100%)**

Table 2. Demographic Characteristics

Variable		HIV+ve (160)	HIV+ malaria (30)	
		No	No	(%)
Age	>50 years	14	2	6.5%
	10- <20	22	4	13.3%
	20- <30	31	8	26.7%
	30- <40	53	10	33.3%
	40- <50	40	6	20%
Gender	Male	62	15	50%
	Female	98	15	50%
Marital status	Single	70	18	60%
	Married	90	12	40%
Occupation	Employed	44	6	20%
	Business	42	9	30%
	Farmers	53	12	40%
	Unemployed	21	3	10%
Education level	Primary	46	10	33.3%
	Secondary	52	10	33.3%
	Tertiary	44	7	23.3%
	uneducated	18	3	10%
Mosquito net use	Use	130	4	10.3%
	Do not use	30	26	89.7%
Alcohol consumption	alcoholic	100	19	63.3%
	Non-alcohol	60	11	36.7%

Table 4: HIV/AIDS serological Status concerning the marital status of the participants

	Negative		Positive	
Ages	**Female**	**Male**	**Female**	**Male**
>50	3(25%)	9(75%)	1(50%)	1(50%)
10-20	11(64.7%)	6(34.3%)	2(50%)	2(50%)
20-30	18(78.3%)	5(21.7%)	6(75%)	2(25%)
30-40	30(69.8%)	13(30.2%)	4(40%)	6(60%)
40-50	21(61.8%)	13(28.2%)	2(33.3%)	4(66.7%)
Total	83(64.3%)	46(35.7%)	15(50%)	15(50%)

Table 3: Sub-regional distribution of HIV/AIDS among studied participants

HIV participants		**Malaria status**	
Address	**No (%)**	**_VE**	**+VE**
Bunyaruguru	8 (5.0)	6	1
Busoga	21(13.2)	13	8
Butare	7 (4.4)	6	1
Comboni	6 (3.8)	6	0
Kabinge	9 (5.6)	7	2
Kanyanga	9 (5.6)	8	1
Kazinga	23(14.4)	19	4
Kigoma	13 (8.1)	11	2
Kyamaminzi	6 (3.8)	5	1
Mashanga	6 (3.8)	6	0
Ndekye	25(15.6)	17	8
Nyakiyanga	14 (8.8)	14	0
Rutoto	9(5.6)	9	1
Others	4 (2,5)	2	2
TOTAL	160	129	31

DISCUSSION

Febrile illness remains a problem in developing countries with a poor resource for malaria diagnosis and management. Poor resources remain a priority health challenge to developing countries especially in hard-to-reach areas where people still get sick, deteriorate and die without getting access to healthcare. In this study, we report a 19% overall prevalence of Malaria in the study population of HIV infected and AIDS patients. This19% is lower than 53.3% reported by Agwu et al, (4) in South Southern Nigeria

town of Ekpoma, 49.83% in South-Eastern Nigeria as reported by Johnbull et al, (7), 25% in South Western Nigeria city of Lagos, (8) and 32% in South Africa as reported by UNICEF, (9). Malaria is known to have the greatest impact on very young children and pregnant mothers. HIV/AIDS unduly affects the young, economically active population. Co-infections with malaria and HIV/AIDS also have major health implications (2). Individuals who live in areas with a high *P. falciparum* parasite rate have about twice the risk of being HIV positive compared with individuals who live in areas with a low *P. falciparum* parasite rate.

The biological basis of Malaria and HIV interactions has already been well established. Briefly, HIV infection induces cellular diminution and early abnormalities of CD4+ T cells, decreases CD8+ T-cell counts and function (with regards to cellular immunity), causes deterioration of specific antigen responses (humoral immunity), and leads to alteration of innate immunity through impairment of cytolytic activity and cytokine production by natural killer cells. Therefore, HIV infection affects the immune response to malaria, particularly premonition in adolescents and adults, and pregnancy-specific immunity, leading to different patterns of disease in HIV-infected patients compared with HIV-uninfected patients. It is estimated that an additional 3 million cases of malaria and 65 000 additional malaria-related deaths annually are due to the impact of HIV (10).

Malaria infection causes an increase in plasma HIV viral load. CD4+ T lymphocytes decline temporarily during clinical malaria episodes in HIV-infected and HIV-uninfected patients (11), and repeated malaria infections are associated with a more rapid decline in CD4+ T lymphocytes (12), signifying that malaria may lead to faster disease progression from HIV to AIDS. HIV infection predisposes to more recurrent episodes of symptomatic malaria (11), and more occurrences of plain or difficult malaria including death in both children and adults (12-15). Generally, patients with HIV respond to standard malaria regimens.

Infection with HIV has been associated with an increased rate of malaria treatment failure may be due to re-infection with new malaria strains, rather than a recrudescence of prior infection (11). Males and females had equal malaria distribution percentages among HIV-infected patients studied (Table 2). In HIV-uninfected women, there is a high risk of placental malaria decrease with each pregnancy. In HIV-infected women, this gravidity-specific pattern is altered, such that multigravidae women carry the same risk of disease as primigravidae women (16). Pregnancy-associated malaria is associated with an increased risk of maternal anemia, intrauterine growth restriction, and delivery of preterm, and low-birth-weight infants (17).

Again from Table 1, Malaria was more prevalent in participants with CD4 counts of 200 -400 cells indicating increasing malaria prevalence in decreasing CD4 cell level of participants. In a cohort study in Kenya, HIV-coinfected women had higher placental parasite densities and higher rates of antenatal malaria transmission than did HIV-uninfected women (17). Maternal antibody to variant surface antigens (VSAs) on malaria-infected erythrocytes plays an important role in pregnancy-related immunity to malaria. Cohort studies in Cameroon show that malaria infection during pregnancy may increase the risk of mother-to-child transmission of HIV (18-19). One potential mechanism for this was evaluated in vitro, where binding of recombinant *P. falciparum* adhesion to chondroitin sulfate A on human placental cells increased HIV-1 replication in those cells, possibly via TNF-alpha stimulation (20).

Although we did not determine the viral load of HIV patients with malaria burden, it is worth mentioning the relationship viral load has with low CD4 patients in Africa. UNICEF (9) acute malaria infection increases viral load. This malaria-associated increase in viral load could lead to increased transmission of HIV and more rapid disease progression. Daily cotrimoxazole prophylaxis among HIV-infected adults is known to have reduced malaria incidence by 80% in Uganda. Although this effect would be expected to vary, depending on the epidemiological setting and patterns of drug resistance, it may be relevant for both pregnant women and adults. The observed malaria distribution pattern in this

study might be due to the geographical location and the weather changes of the area.

Using both the rapid diagnostic test (RDT) and the microscopic diagnostic methods, 15% of malaria patients were females and 25% malaria patients were males. These results are similar to 17% -33% of urban dwellers in Mozambique as having malaria (Holmes et al 21). This could be explained by the fact that Malaria and HIV affect hundreds of people across overlapping geographic distributions especially in western Uganda, and the risk of transmission of both malaria and HIV may be increased due to coinfection with other agents of diseases. It has been observed that HIV-infected people in areas of malaria transmission have more frequent episodes of symptomatic parasitemia and higher parasitemia than those without HIV as quoted by Whitworth *et al* (6). Given the sheer numbers of people living with HIV in western Uganda, an area where malaria transmission is common, there is a concern for a significant public health threat as quoted by Kamya *et al,* (22).

The study participants with both HIV and malaria had low CD4 cell count levels of above 200 and below 400 cells/µl. While those with CD4 cell count below 200cell/µl were 5(16%), those with CD4 cell count between 200-<400 were 25(83.3%). These reduced CD4 cell count observed among malaria and HIV-infected patients agree with Mermin (23) who reports that malaria is associated with the more rapid decline in CD4 cell count. These also tallied with Xio's (24), study who mentioned that the risk of malaria becomes more pronounced with advancing immune suppression, such that at CD4 counts below 200 cells/ml, individuals suffer more than twice the rate of malarial fever as those with CD4 counts above 500 cells/ml. This reduced CD4 cell count could be explained by the fact that HIV infects and depletes CD4+ T lymphocytes, putting patients at risk for other infections. This immune activation, rather than being a reflection of antiviral immunity, may be associated with HIV-1 disease progression. It is also a potential means by which HIV affects disease course and outcome in other infections, such as malaria.

Most participants 79(49%) had CD4 cells between 600-<800 while 10(5%) had CD4 cell count below 200 were 10(5%). There was a lower CD4 cell count among patients with both malaria and HIV when compared to the ones with only HIV. CD4 cell count of 200-<400 had 30 participants suffering from both HIV and malaria, while CD4 above 500 had patients with no malaria. These may indicate that malaria causes an increase in the viral load which leads to the destruction of more CD4cells thus the number of CD4 cells reduce. In HIV-positive patients with CD4 >200 cells/µl, a marked significant increase was obtained when meaning viral load at baseline was determined as suggested by Tatfeng *et al,* (2).

Female were more available 98(61%) than males 15(15%) of them having malaria. HIV could be because women visit the hospital regularly compared to males. Pregnant females are likely to discover their status early because they are more anxious and goes for antenatal care where they are tested for HIV and malaria. On the other hand, males only visit the hospital when they feel sick, thus delaying the chance in which they will know their HIV/AIDS status. Also during delivery, women are prone to HIV and malaria if they receive a blood transfusion.

The study also showed that the mosquito nets were being distributed maximally and 131(83%) out of 160 participants were using them and only 27(17%) participants were not using them. These also showed that the nets can be relatively effective in preventing the spread of malaria because the participants infected with malaria were the ones not on nets. The age of participants with the highest number of HIV and malaria co-infection was 30-40 years which had 10 (33.3%) a number which was lower than 56.6% of the same age group reported by Agwu *et al*, (4). This could be because this is the age where community members have families and work late into the night outside mosquito nets.

Most participants who took alcohol had a higher prevalence of HIV and malaria at 19(63.3%) when compared to the ones who did not take alcohol at 11(37.7%). This could be due to the known intoxicating effect of alcoholism whereby people under the influence of alcohol can stay outside the mosquito nets outside their houses at social events such as religious, marriage, burial, and patties. Uganda faces multiple health

problems and the average life expectancy for the country's 33 million people is 53 years. Amongst adults aged 15 to 49, 6.5% carry HIV, (25).

HIV and malaria have similar global distributions, with the majority of those affected living in sub-Saharan Africa, the Indian subcontinent, and Southeast Asia. Thus they are synergistic and overlapping in that HIV-infected adults and children have a higher rate of malaria. Advanced immune suppression is also associated with an increased incidence of malaria. Malaria episodes are associated with a short-term increase in viral load and a decrease in CD4 cell count. Also, malaria could accelerate HIV disease progression and facilitate HIV transmission (25). HIV is associated with increased placental malaria and poor birth outcomes. HIV infection could disrupt immune responses to malaria and may increase the incidence and severity of malaria. Routine intervention for HIV may impact the incidence of malaria in that therapies for each infection may impact upon the other, leading to unanticipated effects on drug efficacy or toxicity (25). The control of malaria parasitemia is immune-mediated, and this prevents most malarial infections from becoming clinically apparent in semi-immune adults in endemic areas. The interaction between HIV and malaria has proved to be remarkably subtle (26). The increasing incidence of HIV-associated febrile illnesses leads to increased use of antimalarial and may have encouraged the development of resistance to antimalarial drugs or inadequate clearance of parasites that escape drug action during treatment (27).

The human immune response to malaria and HIV shows the influence on the clinical course of the other. Many other types of infections are associated with at least a transient increase in HIV viral load. Hence, it is logical to expect malaria to do the same and potentially accelerate HIV disease progression (2). Available data have shown a greater prevalence of parasitemia episodes in HIV-infected individuals, which increases with falling CD4-cell count. Besides, HIV-infected adults are more likely to develop malaria, and this risk becomes more pronounced with advancing immunosuppression (28). In those infected with HIV, susceptibility to malaria and parasitemia increases as immune responses fail. Clinical malaria is more common, particularly in less immune people. Acute falciparum malaria increases transiently HIV viral concentration. In pregnant women, particularly multigravida, HIV-associated immunosuppression contributes to more frequent and severe malaria, anemia, placental malaria, low birth weight, and poor infant survival. Malaria contributes to increased HIV replication, with possible disease progression and increased risk of mother-to-child transmission of HIV. Uganda has demonstrated that HIV infection approximately doubles the risk of malaria parasitemia and malaria in non-pregnant adults, and that increasing HIV-immunosuppression is associated with higher density parasitemia (28)

It is not clear why participants from different locations attending the hospitals had different malaria and HIV distribution as shown in Table 4. However, this information will be useful in generating a surveillance map vis-à-vis the distribution overlap of the two diseases in a small sub-region like the studied area within major malaria and HIV endemic region of Bushenyi and Kasese districts of Uganda. Future studies may wish to examine the impacts of mixed racial or geographical aspects of the dynamics of HIV/AIDS and malaria transmission in resource-limited communities.

In conclusion, malaria co-infection in HIV was 19%. The prevalence of HIV and malaria is 33.3% high among working-class people of ages 30-40 years and this could be linked to their occupation such as working too late at night which exposes them to mosquito bites. Immunosuppression or CD4 level inversely impacted the malaria distribution. Thus low CD4 was associated with high malaria distribution and high malaria distribution was common with participants with low CD4 count. Overall, HIV was high among Malaria patients and malaria was high among HIV-infected and AIDS patients. Mosquito net usage was effective in malaria control among those who used them but was ineffective among some age groups characterized by constant stay outside the mosquito net due to alcoholism and a variety of

social events which makes people stay outside the nets at nights. Such social events were fully discussed. From the above conclusion, the following are recommended: there is a need for sensitizations of the community about the transmission modes emphasizing prevention, early detection, and treatment of Malaria. All HIV-infected individuals should be offered insecticide-treated bed nets in addition to other standard preventive measures. Patients who test HIV positive should also be tested for malaria and if infected with malaria they should be treated. Patients who have low CD 4 cell count should be given antimalarial on top of another drug to prevent further reduction of CD4 cells. Malaria-associated anemia often requires blood transfusions, blood should be adequately screened for HIV and malaria to prevent further spread of both diseases.

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