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Comparative Study on the Haematological Characteristics of Malaria Infected and Malaria Non-Infected Persons Referred to Art/HIV Laboratory, Abia State University Teaching Hospital, Aba, Abia State.

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ABSTRACT

People infected with HIV/AIDS frequently have hematological derangements which include anaemia, Leucopenia, and Thrombocytopenia. In this study, hematological parameters between malaria positive(+) and negative(--) HIV infected persons referred to ART/HIV laboratory, Abia State University teaching hospital, Aba were compared. Blood samples collected for haematological assays were run using an automated full blood counter and malaria parasitaemia was determined by blood smear microscopy. A total of 490 adults living with HIV parasites were examined. Of these 373(76.1%) were found to have malaria while 117(23.3%) had no malaria infection. Haematological analysis of blood samples showed higher mean values of RBC ($\times 10^{12}$ /µL)(4.66±4.28 for (--)/3.80±2.30, for (+),WBC($\times 10^{9}$/µL)(5.39±1.68 for (--)/5.35±3.61 for (+), HB(g/dl)(12.16±2.41 for (--)/10.44±2.09 for (+),HCT(%)(36.42±6.71 for (--)/32.45±15.82 for (+),MCH(pg)(28.67±3.76 for (--/28.32±4.38 for (+), MCHC(g/dl)(33.28±1.38 for (--)/32.64±2.37 for (+), PLT(x10^{9}/µL)(233.56±123.26 for (--)/227.20±93.98 for (+)LYM(%)(50.83±56.78 for (--)/41.53±13.67 for (+), NEUT(%)(39.68±14.66 for (--)/37.98±19.19 for (+), for malaria uninfected persons. Lower mean values of MCV (FL)(85.54±10.03 for (--)/86.63±11.09 for (+), MXD(%)(16.08±9.93 for (--)/23.04±28.83 for (+) were observed in malaria uninfected persons. The variation seen in the mean

values of RBC, HB, HCT, MCHC and MXD among malaria infected and malaria non-infected persons was found to be statistically significant (with t values 2.817, 6.946, 2.646, 2.774 and-3.975 respectively), while the differences seen in the mean values of WBC, MCV, MCH, PLT, LYM and NEUT among malaria infected and malaria non-infected persons was found to be statistically insignificant (with t value 0.138, -0.950, 0.785, 0.590, 0.082 and 1.011 respectively). These data suggest that malaria may not be the only cause of hematological abnormalities in HIV infected persons studied. There is need for further investigations to identify and treat other potential causes.

Key words: Malaria, HIV, Haematological, Non-pregnant.

Introduction:

The two most serious and common infections, in Africa have remained malaria and the Acquired Immunodeficiency Syndrome (AIDS). Malaria and HIV/AIDS constitute global public health problems, resulting in considerable morbidity and mortality (Diallo *et al.,* 2004). Together, they accounted for over 3 million deaths in 2007 (WHO, 2008, UNAIDS, 2007), and millions more are adversely affected each year.

Malaria remains the most serious and widespread protozoan infection of human. Over 40% of the world's population is at risk of contracting malaria (WHO, 1996). It is a life threatening infection with an estimated 216 million cases and 655,000 deaths per year globally and approximately 91% of these deaths occur in sub-Sahara Africa (WHO, 2011). Malaria is a mosquito borne infectious disease of humans, caused by Eukaryotic protists of the genus *Plasmodium*. It is widespread in tropical; and subtropical regions, mostly sub-Sahara Africa and Asia due to the significant amounts of rainfall, consistent temperature and high humidity along with the availability of stagnant waters in which their larvae mature. Malaria results from the multiplication of malaria parasites within red blood cells, causing symptoms that may include fever, headache and in severe cases, progressing to coma and death. Four species of *Plasmodium* infect humans, but severe

malaria is caused by *Plasmodium falciparum* .Malaria infection caused by *Plasmodium vivax, P. ovale,* and *P. malariae* is usually milder and rarely fatal. Although transmission of malaria can be reduced by preventing mosquito bites, using insecticide treated nets, insect repellent creams, or by other mosquito control measures,such as spraying insecticides inside the house and draining standing water where mosquitoes lay their eggs. The challenge of producing a widely available Vaccine, which provides high level of protection for a sustained period, is yet to be met (Kilama and Ntoumi, 2009).

HIV infection in humans is considered Pandemic by the World Health Organization; nevertheless, complacency about HIV may play a key role in HIV risk (CDC, 2010). From its discovery in 1981-2006, AIDS killed more than 25million people (J.U.N.P., 2006). HIV infects about 0.6% of the world's population (J.U.N.P., 2006). In 2009, AIDS claimed an estimated 1.8 million lives, down from a global peak of 2.1 million in 2004 (J.U.N.P., 2010). A disproportionate number of AIDS deaths occur in sub-Saharan Africa, retarding economic growth and increasing the burden of poverty (Greener, 2002). In Nigeria, the prevalence of HIV has been on steady increase, from 1.8% in 1991 to 3.8% in 1993, 4.5% in 199 and 5.8% in 2001 (Federal Ministry of Health, 2001). A complete blood count (CBC) or full blood count (FBC) is a test panel requested by a doctor or other medical personels, it gives information about the cells in a patient's blood. Hematological abnormalities that have been reported to be associated with malaria infection include Anaemia, Thrombocytopenia, Leucopenia, Eosinophilia and Leucocytosis (Facer, 1994), (Adejuyigbe and Oninla, 2003). On the other hand, cytopenia, resulting from the myelosuppressive effects of opportunistic infection and other complications of AIDS are common in HIV/AIDS patients.

Nigeria is highly burdened with malaria and HIV, yet, the impact of this co-infection on the hematological parameters of HIV-infected patients is not well understood. More information is needed to guide the simultaneous treatment of both diseases. This study aims at investigating the hematological changes that may occur in acute malaria infection of HIV-infected patients. This will go a long way in alerting and updating

informations for physicians, who take care of HIV-infected patients, reducing the high mortality and morbidity, associated with the co-infection, as we continue to work toward getting a lasting solution for HIV/AIDS.

Materials and Methods

Study Area

The study was conducted in Aba metropolis and precisely in Antiretroviral/HIV (ART/HIV) Laboratory, Abia State University Teaching Hospital (ABSUTH), Aba, Abia, state, Nigeria, from June to September, 2011. ART/HIV Laboratory, ABSUTH was founded 6 years ago by the former American President, George Bush under President's Emergency Programme for AIDS Research (PEPFAR), and is currently funded by Strengthening Integrated Delivery of HIV/AIDS Services(SIDHAS), with the aim of strengthening and integrating health service delivery for the HIV infected. HIV patients are refered to ART/HIV Laboratory on diagnosis. In this laboratory, routine checkups and administration of antiretroviral drugs are done for them, free of charge.

Aba, (5°07'23''N, 7°22'08E) is a very busy economic town in Abia State. It attracts people of all social backgrounds. It is commonly called the 'Japan of Africa', because of its industrial nature. Unfortunately, the industrial and economic revolution in the town has created a lot of social problems like child defilement, rape, prostitution and armed robbery. The area has rainy and dry seasons, running from mid-March to October and from November to mid- March respectively. The town has stagnant water pools around the houses, especially in unpopular quarters, during the rainy season, poor waste disposal system and overcrowded human population is the order of the day in Aba. Malaria is endemic in Aba and most parts of Nigeria, with high transmission rate. Previous workers have observed that Malaria in this part of the country is predominantly due to *Plasmodium falciparum*. Aba therefore, is an ideal site to investigate HIV/Malaria co-infection.

Ethical Clearance

The research was examined, modified and approved by the Ethical Clearance Unit of the Abia State University Teaching Hospital (ABSUTH), Aba, Abia, state, Nigeria

Subjects/Inclusion Criteria:

Those included in this study were non pregnant individuals, confirmed to be living with HIV/AIDS, who were not on any malarial treatment during the study period.

Method

The subjects were first confirmed to be HIV infected, using a double Enzyme Immuno Assay (EIA) of WHO approved immunocomb HIV 1 & 2 KITS (organics, Israel) and Determine HIV 1 & 2 (Abbott lab, Minato-ku, Tokyo, Japan) both are qualitative and differential tests for the detection of antibodies to HIV 1 & II in human serum or plasma. After providing written consent, social demographic date (Age and Sex) and anti-retroviral drug history were recorded.

Using sterile plastic syringes, 3mls of venous blood sample was aseptically collected from each participant into EDTA vacutainer tubes for laboratory investigations. Thick film was prepared using each participant's blood sample within 30 minutes of collection, following WHO (1999) procedures. Another portion of the blood sample was used for full blood counts, using sysmex automated full blood counter (Ike *et al.,* 2010)

Statistical Data Analysis

Data collected were analyzed, using SPSS (Statistical Package for Social Science) 17.0. The descriptive statistics were used to compute the results. The t-test was used to compare infections by gender and subject administered with Antiretroviral drugs and those not given Antiretroviral drugs. Chi-square (χ^2) was also used to test the

independence of infected subjects according to age groups. P-values <0.05 were considered significant.

Results

Of the 490 non-pregnant adult HIV infected persons sampled, 373(76.1%) were infected with malaria while 117(23.88%) were not. The females recorded a higher percentage infection rate 281(81.21%) than the males 92 (63.89%). The difference in the sex-related infection rate was found to be statistically significant (X^2cal=15.852; df=1,p<0.05). Prevalence of malaria parasite with respect to sex is shown in figure I.

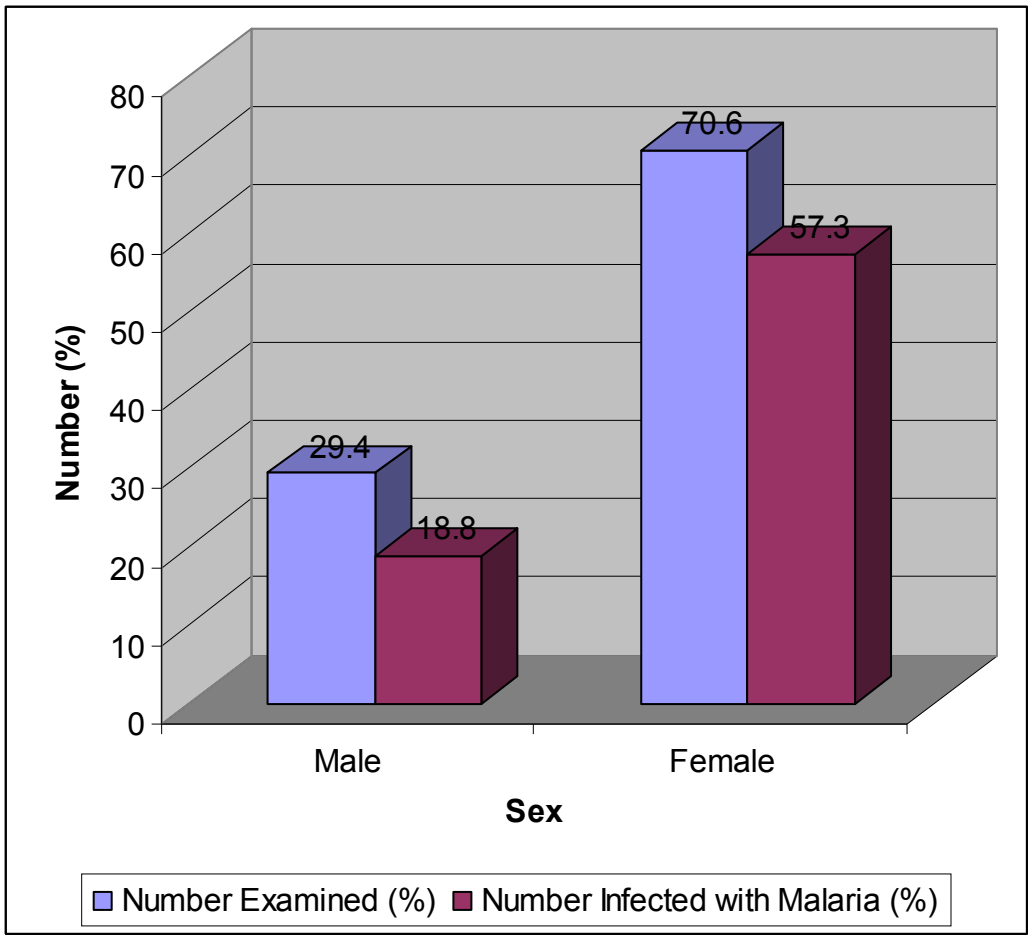

Figure I: Sex-Related Prevalence of Malaria among HIV Infected Persons Examined

Table I: Independent Samples Test for MP Infected and Not Infected Subjects (Mean ± SD)

Parameter	Infected n = 373	Not Infected n = 117	t-value	p-value	Remark
WBC (×10^9/µL)	5.35 ± 3.61	5.39 ± 1.68	0.138	0.890	NS
RBC (×10^{12}/µL)					
HB (g/dL)	3.80 ± 2.30	4.66 ± 4.28	2.817	0.005	S
HCT (%)	10.44 ± 2.09	12.16 ± 2.41	6.946	0.000	S
MCV (FL)	32.45 ±	36.42 ± 6.71	2.646	0.008	S
MCH (pg)	15.82	85.54 ± 10.03	-0.950	0.343	NS
MCHC (g/dL)	86.63 ±	28.67 ± 3.76	0.785	0.433	NS
PLT (×10^9/µL)	11.09	33.28 ± 1.38	2.774	0.006	S
LYM (%)	28.32 ± 4.38	233.56 ±	0.590	0.555	NS
MXD (%)	32.64 ± 2.37	123.26	1.756	0.082	NS
NEUT (%)	227.20 ±	50.83 ± 56.78	-3.975	0.000	S
	93.98	16.08 ± 9.93	1.011	0.313	NS
	41.53 ±	39.68 ± 14.66			
	13.67				
	23.04 ±				
	28.83				
	37.98 ±				
	19.19				

The means of RBC(4.66±4.28;3.80±2.30)×10^{12}/µL,HB(12.16±2.41;10.44±2.09)g/dl, HCT(36.42±6.71;32.45±15.82)%, MCHC(33.28±1.38;32.64±2.37)g/dl values were higher in malaria non-infected persons than in malaria infected. The difference in RBC, HB, HCT and MCHC of malaria infected and malaria non-infected was statistically significant with t values 2.817,6.946,2.646 and 2.774 respectively. The means of WBC(5.39±1.68;5.35±3.61)×10^9/µL, MCV(85.54±10.03)FL and MCH(28.67±3.76;28.32±4.38)pg, PLT(233.56±123.26;227.20±93.98)×10^9

Table II: Independent Sample Test for Malaria Infected Subjects by Sex. (Mean ± SD)

Parameter	Male n = 92	Female n = 281	t-value	p-value	Remark
WBC	5.34 ± 2.16	5.35 ± 3.98	-0.019	0.985	NS
RBC	4.00 ± 0.92	3.74 ± 2.60	0.944	0.346	NS
HB	10.92 ± 2.38	10.28 ± 1.96	2.351	0.020	S
HCT	33.30 ± 6.90	32.17 ± 17.80	0.596	0.551	NS
MCV	85.10 ±	87.13 ± 11.27	-1.528	0.127	NS
MCH	10.40	28.54 ± 4.39	-1.706	0.089	NS
MCHC	27.64 ± 4.31	32.69 ± 2.55	-0.735	0.463	NS
PLT	32.48 ± 1.70	225.50 ±	0.611	0.541	NS
LYM	232.40 ±	92.67	-1.774	0.070	NS
MXD	98.21	42.25 ± 13.63	-0.854	0.393	NS
NEUT	39.34 ±	23.77 ± 32.23	0.718	0.473	NS
	13.63	37.57 ± 19.87			
	20.81 ±				
	13.98				
	39.22 ±				
	16.98				

S = Significant; NS = Not Significant

The means of RBC(4.00±0.92;3.74±2.60),HB(10.92±2.38;10.28±1.96), HCT(33.30±6.90;32.17±17.80);PLT(232.40±98.21;225.50±92.67) and NEUT(39.22±16.98;37.57±19.86) values were higher in malaria infected males than the malaria infected females. Whereas WBC(5.34±2.16;5.35±3.98),MCV(85.10±10.40;87.13±11.27) MCH(27.64±4.31; 28.54±4.39), MCHC(32.48±1.70;32.69±2.55), LYM(39.34±13.63; 42.25±13.63) and MXD(20.81±13.98;23.77±32.23) values were higher in malaria infected females than

the malaria infected males. The differences were not statistically significantly, except the difference in HB, with t value (2.351).

Table III:

(Mean ± SD)

Parameter	Absent n = 218	Present n = 155	t-value	p-value	Remark
WBC	5.40 ± 3.36	5.28 ± 3.96	0.354	0.724	NS
RBC	3.85 ± 2.94	3.73 ± 0.81	0.525	0.600	NS
HB	10.14 ± 2.14	10.85 ± 1.95	-3.249	0.001	S
HCT	32.19 ±	32.81 ± 5.63	-0.375	0.708	NS
MCV	20.16	90.25 ± 12.95	-5.190	0.000	S
MCH	84.05 ± 8.70	29.34 ± 5.10	-3.677	0.000	S
MCHC	27.59 ± 3.64	32.89 ± 1.70	-1.681	0.094	NS
PLT	32.47 ± 2.74	232.41 ±	-0.902	0.368	NS
LYM	223.50 ±	102.52	0.341	0.734	NS
MXD	87.45	41.25 ± 12.96	3.175	0.002	S
NEUT	41.74 ±	18.18 ± 11.91	-2.196	0.029	S
	14.19	40.40 ± 14.87			
	26.50 ±				
	35.99				
	36.25 ±				
	21.62				

Out of the 373 persons infected with malaria parasite, Antiretroviral drugs were administered to 155 persons, while 218 were not on antiretroviral drugs. HB, MCV, MCH, MXD and NEUT with t-values – 3.249, -5.190, -3.677, 3.175 and -2.196 respectively showed a significant difference in the means of those given the drugs and those not given.

DISCUSSION

Haematological anomalies are considered a hallmark of malaria, and reported to be most pronounced in *Plasmodium falciparum* infections (Richards *et al.*, 1998). In this study, the mean values of hematological parameters appeared lower in participants, having malaria parasitemia when compared with those not infected. However, not all the differences were statistically significant.

The mean values of RBC, HB, HCT, MCHC, and MXD of malaria parasitized subjects were significantly different from those of the non-infected subjects. This finding is consistent with the previous findings by Erhabor *et. al.* (2006) and Nkwo- Akenji *et al.,* (2008), who found malaria to be significantly associated with Anaemia in HIV/AIDS infected persons. The pathogenesis of anaemia in malaria parasitized patients are complex and multifactorial. They are thought to be as a result of hemolysis of parasitized RBCs, worsened by removal of parasitized red cells, depressed and infective erythropoiesis (Erhabor *et al.,* 2006)

The mean lymphocyte and total white cell count in malaria positive participants were not significantly different from those of malaria negative participants at ($p > 0.005$). These observations also agreed with previous findings by Erhabor *et al.,* (2006), showing that malaria in HIV – infection may not be associated with leucopenia. The mean platelet count in malaria positive participants was not significantly different from that in malaria negative participants ($p > 0.05$). This was not in agreement with the previous finding that mean platelet count in malaria parasitized HIV/AIDS patients was significantly lower as compared to non-parasitized controls (Erhabor *et al.,* 2006)

Several other factors apart from malaria could contribute to hematological parameter in people living with HIV/AIDS. These include nutrition and intake of nutrients like vitamin B_{12}, iron, folic acid, and the use of antiretroviral drugs, some of these may impact

hematological parameters. Opportunistic infections may also modify the immune response and thus, modify WBC and lymphocyte counts as well as therapies for these other opportunistic infections. The relative roles of each of these factors could vary from population to population. Findings from this study suggest that, apart from malaria, other factors may also play significant role. The use of multivariate analysis, controlling for age, gender and antiretroviral use as well as relatively large sample size were strengths of this study.

These findings indicate that malaria may not be the only cause of hematological disorders in these patients, thus, findings of hematological abnormalities or derangements should not be assumed to be caused by malaria. More investigations are needed to establish the origin of these abnormalities and the relationship with malaria and malaria treatment, to aid the investigation and management of hematological abnormalities in people living with HIV/AIDS.

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