Malaria Parasites Burden at Various Stages of Human Immunodeficiency Virus (HIV) Infection

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ABSTRACT

Background: Studies have shown that there are more incident and severe occurrences of malaria among HIV infected individuals, and markers of HIV disease sequence exacerbate during acute malaria. This study was designed to evaluate the malaria parasites burden on various stages of HIV among infected subjects. Total of 116 co-infected subjects were used and compared with HIV mono-infected and apparently healthy subjects. **Method:** About 8mls of venous blood sample was drawn from each subject. Malaria parasite density was determined by blood film examination as well as assay of some immune-cellular and biochemical parameters using Sysmex Kx-21N automated Haematology Analyzer. **Result:** The study showed that HIV stage I present the highest prevalence of co-infection (22.4%) whereas stage III had the least prevalence (3.0%) of co-infection. The malaria parasites density was highest in stage III with mean value of $805.00\pm589.53/\mu l$. This mean value was higher ($p<0.05$) than the least mean value ($307.33\pm222.65/\mu l$) from stage I subjects. Stages I and II showed that intake of ART reduces parasites burden. However, the reverse was the case for stage III and IV subjects. Stage III proved to have the highest mean granulocytes values ($70.45\pm27.31\%$) and the least total WBC count ($5.05\pm1.87\times10^9 l$). ART intake significantly reduced lymphocytes especially in stage I ($36.12\pm21.07\%$) and III ($1.00\pm0.00\%$) subjects. Stage III co-infected subjects had significantly low mean PCV ($22.50\pm2.67\%$). **Conclusion:** High malaria parasites density and reduced WBCs as well as PCV observed in stage III co-infected subjects could increase the risk of negative disease outcome. Hence, concerted efforts are required to prevent disease progression at this stage.

INTRODUCTION

Malaria is a disease associated with Plasmodium spp. It is transmitted by an infected female *Anopheles* mosquito during blood meal (1). Of all the species of plasmodium, *P. falciparum* is the common cause of severe malaria (1,2). Symptoms of malaria may include fever, feeling tired, vomiting, and headaches. Seizures, coma, or death may be seen in severe case (2).

Human immunodeficiency virus infection is a pathological state occasioned by infection with the human immunodeficiency virus (3-5). Initial infection may be asymptomic or with brief period of influenza-like illness (6). The virus interferes with the immune system as the infection progresses, this increases the risk of opportunistic infections, and tumors that rarely affect immuno-competent individuals (6).

HIV infection is pandemic with greater prevalence in sub-Saharan Africa and Asia (7). These regions are also the hub of malaria (8). Studies have shown that there are more incident and severe episodes of malaria among HIV infected individuals, and parameters of HIV disease progression worsen in individuals during acute malaria episodes (9). Each of malaria and HIV disease alone causes serious morbidity and mortality, and a threat to public health in these regions.

This research was designed to determine the burden of malarial parasites on the various clinical stages of HIV and the disease severity of such co-infection.

MATERIALS AND METHOD

Study Location

The study area was Edo Central. This area is located between latitude '6^0 10 and 6^0 45'

north of the equator and between longitudes 6^0 10' and 6^0 30' east of the Greenwich Meridian (10).

Research Design

This was a cross sectional study. Known HIV subjects were used for the study. The recruitment of HIV infected participants was by simple random sampling method. The control subjects were apparently healthy subjects within the study community.

Sample Size

The test samples were obtained from 116 HIV/malaria co-infected subjects and 92 apparently healthy subjects between 7 to 63 age group visiting the HIV clinic at General Hospital, Uromi

The sample size was calculated using the formula:

$$N = Z^2pq / D^2 \qquad (11)$$

- Where N = sample size, Z = standard deviation (1.96), p = prevalence, q = 1-p and   D = degree of freedom (0.05), and a 4.6% HIV prevalence for Edo state (12).

Extrapolation from method

$$N = \frac{(1.96)^2 \text{ x } 0.046 \text{ x } (1- 0.046)}{(0.05)^2}$$

$$\frac{3.8416 \text{ x } 0.046 \text{ x } 0.954}{0.0025}$$

$$\frac{0.1686}{0.0025}$$

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To increase reliability of result, 116 test samples were collected.

Selection Criteria: Patients' form containing clinical details where used to rule in/out study subjects following the criteria below;

Inclusion Criteria: Only HIV subjects positive to malaria infection were recruited as test subjects for the study. Apparently healthy subjects were recruited as control.

Exclusion Criteria: Patients with clinical illness (condition) other than malaria and HIV were excluded from the study. Patients' form containing clinical details where used to rule out participants with other common infectious agents.

Ethical Approval

Ethical approval was obtained from the ethics and research committee of Ambrose Alli University, Ekpoma and informed consent of the patients was sought for before sample collection.

Sample Collection

About eight milliliters of venous blood was collected from each patient. Three milliliter (3mls) was dispensed into EDTA anticoagulant bottle for assessment of hematological and cellular immune parameters as well as blood film. Five milliliters was dispensed into lithium heparin bottle for biochemical assay. These samples were immediately analyzed in the Laboratory Department of General Hospital, Uromi. In collecting samples, standard precautionary measures were followed strictly

Malaria Parasite Examination

Malaria parasites examination was by thick blood film according to Cheesbrough(13).

Malaria Parasitemia by Blood Film Examination

A thick blood film stained with giemsa was examined, 200 or 500 white blood cells were counted and the number of malaria parasites were also counted. The number of parasites per microlitre (parasites density) was calculated as recommended by *Denis et al.*, (14).

That is: number of malaria parasites/
          number of WBC counted x Assumed WBC count (10000/µl)

Immuno-cellular parameters, PCV and Biochemical Assay

Immuno-cellular parameters and PCV were quantified using Sysmex Kx-21N automated Haematology Analyzer. Biochemical analysis was carried out using spectrophotometric methods; Serum total protein was processed by Biuret Method as described by Johnson (15); Serum Albumin was determined by Bromocresol Green method as described by Daumas *et al.*, (16); Total cholesterol and Triglyceride were estimated by the Enzymatic method of Nader and Warnick (17), while Glucose oxidase method was used to estimate serum glucose as described by David (18).

Data Analysis

Data generated was presented in a table form. Data obtained from assayed parameter was statistically analysed using ANOVA (SPSS 20.0). Values were expressed as Mean ± SD. A p-value of ≤ 0.05 was considered as significant in all statistical analysis.

RESULTS

This study was carried out to evaluate malaria parasites burden on the various

stages of HIV among infected subjects in Uromi.

Table 1, demonstrate the distribution of subjects across the clinical stages; 120 (44.7%) were in stage I category with 92 on ART, stage II had 48 (18%) subjects with 44 on ART, stage III were 52 (19.3%) 48 on ART while IV were 48 (18%) with 32 on ART.

Table 1: Distribution of Co-Infected Subjects across HIV Stages of Infection

HIV STAGES	MP&HIV /ART No (%)	MP&HIV/ NOART No (%)	HIV/ART	HIV/NOART	TOTAL
Stage I	44 (16.4)	16 (6.0)	48	12	120
Stage II	20 (7.5)	04 (1.5)	24	00	48
Stage III	04 (1.5)	04 (1.5)	44	00	52
Stage IV	16 (6.0)	08 (3.0)	16	08	48
Total	84(31.4)	32(12.0)	132	20	268

KEY: MP&HIV/ART=HIV-Malaria co-infected subjects on Anti-retroviral drug, **MP&HIV/NOART**= HIV-Malaria co-infected subjects not on Anti-retroviral drug, **HIV/ART=** HIV mono-infected subjects on Anti-retroviral drug, **HIV/NOART**= HIV mono-infected subjects not on Anti-retroviral drug.

Considering the distribution and comparison of mean parasitic loads across the four HIV stages as tabulated in table 2, the least mean parasites density (307.33 ± 222.65/µl) was seen in stage I with stage III showing the highest mean value (805.00 ± 589.53/µl). The mean parasitic load for stages II, III, and IV were higher ($p<0.05$) than the mean parasitic load in stage I.

Table 3 display a detailed comparison of mean parasites density between the various HIV stages across the ART groups. Among the ART compliant, stage I showed the lowest mean parasites density (250.00 ± 152.18/µl) which was significantly ($p<0.05$) lower than the mean value in the other three stages. The highest mean value (1450.00 ± 0.00/µl) was seen in stage III and was higher ($p<0.05$) than the mean value in the other three stages. The non ART subjects had different distribution. The highest mean parasites density (1300.00 ± 0.00/µl) was seen in stage II and it was statistically significantly higher than the mean values in the other three stages. Stage IV had the lowest mean parasites density (120.00 ± 0.00/µl).

Comparing the mean parasitic load of corresponding HIV stage across the two ART status, it was observed that the differences between the mean values were statistically significant except for stage I which showed no significant difference.

Table 2: Malaria Parasites Density at the various Stages of Infection

PARAMETER	STAGE I n=60	STAGE II n=24	STAGE III n=8	STAGE IV n=24	F-value	P-value
PARASITE LOAD(count/µL)	307.33 ± 222.65^{a}	700.00 ± 579.14^{b}	805.00 ± 589.53^{b}	568.33 ± 320.58^{b}	4.88	0.00

P>0<0.05
KEY: Values with different superscript are statistically significantly different (P<0.05)

Table 3: Malaria Parasites Density of Subjects on ART

PARAMETER	ART STAGE I n=44	NON ART STAGE I n=16	ART STAGE II 20	NON ART STAGE II n=4	ART STAGE III n=4	NON ART STAGE III n=4	ART STAGE IV n=16	NON ART STAGE IV n=8	F-value	P-value
PARASITE LOAD(Count/µL)	250.00 ± 152.18^{a}	465.00 ± 357.59^{ae}	580.00 ± 361.31^{bde}	1300.0 ± 0.00^{c}	1450.00 ± 0.00^{c}	160.00 ± 0.00^{ad}	792.50 ± 555.53^{b}	120.00 ± 0.00^{a}	8.24	0.00

P>0<0.05
KEY: Values with different superscript are statistically significantly different (P<0.05). **ART**= Anti-retroviral drug users, **Non-ART**= Non anti-retroviral drug users.

Table 4 demonstrates the impact of malaria co-infection on the immune cells. Stage III of co-infected group demonstrates the highest mean granulocyte value while the lowest was identified in stage II with $53.60\pm27.86\%$. The difference was not significant. The peak WBC count was seen in stage II ($12.91\pm10.11X10^{9}$/l) whereas stage III gave the least ($5.05\pm1.87 X10^{9}$/l) and the difference was statistically significant.

The nutritional indices of study population across the four various HIV stages were illustrated in table 5. The least mean PCV ($22.50\pm2.67\%$) was seen in stage III of co-infected group and was lower (p<0.05) than the mean PCV value of all other stages of the co-infected group. Comparing related stages between the two sero-positive groups; **PCV**: stage I of co-infected group revealed significantly lower mean value than its counterpart in mono-infected group. **Glucose concentration**: stage I co-infected group was lower (p<0.05) than its counterpart in mono-infected group. **Total protein concentration**: Stage I of co-infected group had significantly lower mean total protein

concentration than its equivalent in mono-infected group while Stage IV of HIV co-infected group had significantly higher mean total protein than its equivalent in HIV mono-infected group. **Albumin**: mean concentration of 4.27+0.65g/dl and 4.60+0.32g/dl were seen in stages II and IV amid co-infected group respectively which were higher (p<0.05) than their equivalent in mono-infected group. **Total cholesterol**: Stages I and II of co-infected group had significantly lower mean values than their corresponding stages among mono-infected group. **Triglyceride concentration**: Co-infected group had higher (p<0.05) mean values in stages I and II than their equivalent in mono-infected group.

Table 4: Immuno-cellular Status of Study Subjects with Respect to HIV Stages

PARAMETERS	Control n=92	HIV&MP STAGE I n=60	HIV STAGE I n=60	HIV&MP STAGE II n=24	HIV STAGE II n=24	HIV&MP STAGE III n=8	HIV STAGE III n=44	HIV&MP STAGE IV n=24	HIV STAGE IV n=24	F-value	P-value
CD4 (cells/mm^3)	928.39±179.89[a]	**828.07±369.93[b]**	697.87±163.27 [d]	**425.33±398.76[cg]**	467.83±15.68[c]	**276.00±68.42[fg]**	267.18±40.51[f]	**116.17±18.68[e]**	64.50±50.66[e]	88.02	0.00
LYMP (%)	29.93±14.64[a]	**41.13±23.44[b]**	42.30±22.73[b]	**38.66±24.87[a][b]**	42.78±24.60[b]	**24.70±15.33[ac]**	39.30±20.68[bc]	**26.68±4.66[a]**	48.96±17.92[b]	4.83	0.00
MON (%)	5.35±3.12 [a]	**5.06±4.35[a]**	7.78±5.73[bc]	**7.73±3.52[bd]**	6.75±4.52 [ab]	**4.85±1.97 [ab]**	6.66±5.07 [ab]	**4.50±3.51[a]**	10.00±4.96[cd]	4.31	0.00
GRAN (%)	64.71±16.46[a]	**53.80±23.78[b]**	49.90±26.65[b]	**53.60±27.86[b]**	50.63±27.53[b]	**70.45±27.31[acd]**	54.03±22.84 [bd]	**68.81±5.55[a]**	41.03±22.25 [b]	5.38	0.00
TWBC (X10^9/l)	6.46±2.42 [ad]	**8.16±4.79 [ac]**	8.74±4.53 [ag]	**12.91±10.11[b]**	12.88±10.12[b]	**5.05±1.87[aef]**	13.06±11.05[b]	**9.71±4.02[bc][eg]**	4.41±1.45[d][f]	6.88	0.00

P>0<0.05

KEY: Values in the same row with different superscript are statistically significantly different (P<0.05). **CD4**= Cluster of differentiation 4, **LYMP**= Lymphocyte, **MON**= Monocytes, **GRAN**= Granulocyte, **TWBC**=Total white blood cell count, **HIV&MP**= HIV-Malaria co-infected subjects, **HIV**= HIV mono-infected subjects.

Table 5: Some Nutritional Indices of Study Subjects.

PARAMETERS	Control n=92	HIV&MP STAGE I n=60	HIV STAGE I n=60	HIV&MP STAGE II n=24	HIV STAGE II n=24	HIV&MP STAGE III n=8	HIV STAGE III n=44	HIV&MP STAGE IV n=24	HIV STAGE IV n=24	F-value	P-value
PCV (%)	38.83±2.61 [a]	31.13±6.02 [b]	33.15±4.60 [e]	27.03±8.47 [cf]	29.50±7.24 [bf]	22.50±2.67 [d]	25.67±6.01 [cd]	30.16±3.60 [b]	26.67±4.28 [cdf]	40.05	0.00
GLUCOSE (mg/dl)	72.17±21.98 [a]	61.07±11.83 [b]	85.47±71.17 [cde]	69.33±22.27 [ab]	68.67±7.59 [ab]	55.00±4.28 [ab]	72.73±24.65 [abe]	75.67±17.40 [abd]	67.33±14.28 [ab]	2.36	0.02
TOTAL PROTEIN (g/dl)	7.44±0.70 [a]	7.13±0.85 [bc]	7.55±0.74 [a]	7.63±0.33 [a]	7.63±0.70 [a]	7.20±0.00 [abc]	7.52±0.79 [a]	7.38±0.79 [ab]	6.93±1.13 [c]	3.12	0.00
ALBUMIN (g/dl)	4.04±0.50 [ae]	3.77±0.61 [b]	3.89±0.73 [ab]	4.27±0.65 [de]	3.78±0.64 [ab]	4.20±0.64 [abd]	3.96±0.69 [ab]	4.60±0.32 [cd]	3.82±0.41 [ab]	5.81	0.00
GLOBULIN (g/dl)	3.40±0.86 [ag]	3.37±0.91 [ag]	3.66±1.24 [fg]	3.37±0.42 [acg]	3.85±1.09 [cfh]	3.00±0.64 [adg]	3.55±1.23 [agh]	2.78±0.77 [bde]	3.12±0.89 [ae]	2.88	0.00
TOTAL CHOLESTEROL (g/dl)	139.65±37.98 [a]	148.40±32.91 [ac]	152.93±26.51 [cdgh]	149.00±35.91 [ad]	172.50±15.51 [be]	151.50±0.53 [aeg]	149.73±47.70 [afh]	156.00±24.47 [bcdefg]	155.50±27.43 [bcdegh]	2.66	0.01
TRIGLYCERIDE (g/dl)	75.04±25.29 [adefh]	86.13±33.16 [b]	66.33±20.60 [gh]	80.83±31.17 [bd]	57.67±9.92 [gi]	90.50±33.67 [bdej]	104.45±42.11 [cj]	86.83±23.72 [bdef]	80.83±33.77 [abdef]	8.30	0.00

$P > 0 < 0.05$

KEY: Values in the same row with different superscript are statistically significantly different ($P < 0.05$). **PCV**= Packed cell volume, **HIV&MP**= HIV-Malaria co-infected subjects, **HIV**= HIV mono-infected subjects.

DISCUSSION

This study evaluates the malaria parasites burden on various stages of HIV among seropositive subjects in Uromi, Edo State. From the study, HIV stage I (asymptomatic) presents the highest prevalence of co-infection whereas stage III (symptomatic) had the least prevalence of co-infection. This was in agreement with result obtained by Olusola et al.(19) in a study where they reported that the highest prevalence of malaria parasitaemia was observed among the group that had the highest CD4+ T-cell count and the difference was statistically significant. This may be due to the fact that asymptomatic HIV subjects feel healthy and hence engage in domestic and farming activities which predispose them to mosquito bite. Also they may not have patronized antimalaria drugs as much as those of the symptomatic group (lower CD4+ count). On the contrary, the result obtained by Onyenekwe et al.(20) showed that the prevalence of P. falciparum malaria as co-infection amongst the asymptomatic HIV seropositive group was lower than the symptomatic HIV seropositive group. This may be attributed to the depleted immune status of the symptomatic HIV subjects in their study.

The malaria parasites density from the present study was highest in stage III. The mean value was significantly higher than the mean value obtained from stage I subjects. This difference may be due to depleted immune cellular components among stage III subjects (as seen in the CD4+ count). Stage IV subjects had the second highest parasite density. Comparing malaria parasitemia in stage III and stage IV, this result did not agree with most studies where it has been reported that malaria parasite burden is inversely proportional to CD4+ count (21-22).Similarly, it has been well establishment that CD4+ T lymphocyte cells <200 cells/µl is associated with a higher risk of opportunistic infection (23). The reason for this variation may be due to marked reduction in red blood cell (host cell for malaria parasite) in stage IV and equally higher tendency for emergency medical intervention (including anti-malaria drugs) for stage IV subjects.

Association of parasites density with ART status showed from this study that intake of ART reduces parasites burden as seen among stage I and II. This variation may be due to the anti-retroviral effect of ART. This pattern agrees with the work of Hewitt et al.(24) who reported that HIV viral load is directly proportional to the risk of malaria infection and development of clinical malaria

However, stages III and IV reveals opposite trend between intake of ART and malaria burden. Those on ART had significantly higher malaria parasites density than their non ART counterpart. This discrepancy may be due to frequent intake of anti-malaria drugs by the non ART subjects just to feel better. This may also suggest that intake of ART at these stages can contribute to immune suppression.

Among the co-infected subjects, those in stage III prove to have the highest mean granulocytes values and the least total WBC count. This result obtained in stage III may be attributed to the increased parasites density and possible multiple infections.

Stage III co-infected group in this study had significantly low mean PCV. This could be attributed to the increased parasitemia observed in this category of the study population (25-26). Stages I, II, and III for co-infected subjects had lower (p>0.05) PCV than the mono-infected counterpart. The disparity may be due to haemolytic activity of malaria parasites. However, Stage IV

subjects showed reverse trend. This variation may be as a result of medical intervention such as blood transfusion.

CONCLUSION

Our study focused on malaria burden across the various clinical stages of HIV. The high malaria parasites density and reduced WBCs as well as PCV observed in stage III co-infected subjects could increase the risk of negative disease outcome. Hence, concerted efforts are required to prevent disease progression at this stage, AIDS progression to death may be difficult to reverse due to a combination of nutritional and cellular immunity depletion as reported in this study.

RESEARCH LIMITATIONS

Viral load was not determined and we could not draw a direct correlation between parasites density and viral load for each of the clinical stages. The sampling method (random sampling) could not provide uniform sample size for all the clinical stages which resulted to a biased sample size across the stage

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