

Possible biochemical impact of malaria infection in subjects with HIV co-infection in Anambra state, Nigeria

C.C. Onyenekwe^a, N. Ukibe^a, S.C. Meludu^b, M. Ifeanyi^c, M. Ezeani^c, A. Onochie^d, N. Ofiaeli^e, N. Aboh^b & A. Ilika^f

^aDepartment of Chemical Pathology, ^bDepartment of Human Biochemistry, ^cDepartment of Immunology, ^dDepartment of Community Medicine, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus; ^eHIV Laboratory Unit and ^fVCT Unit, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria

Abstract

Background & objectives: The present study was designed to determine possible contributory impact of malaria infection on some biochemical markers in subjects with HIV co-infection in order to know if they are adverse or protective.

Methods: Participants were recruited at the Voluntary Counseling and Testing Unit, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria and grouped into: (i) Malaria and HIV co-infection group (n = 45); and (ii) HIV infected group without concurrent malaria infection (n = 57). Standard laboratory methods were used for the HIV and *Plasmodium falciparum* antigen screening, malaria parasite density, CD4⁺ T-cell count, packed cell volume, white blood cell count, serum iron and albumin concentrations.

Results: The results showed that serum iron and albumin were significantly reduced and raised respectively in 'Malaria-HIV co-infection group' compared with 'HIV infection group' (p < 0.05 and p < 0.05). A positive association was observed between age and serum iron concentration in malaria and HIV co-infected group (r = 0.580; p < 0.05) while negative associations were observed between PCV and serum iron (r = -0.388; p < 0.05) and between CD4⁺ T-cells and serum iron concentration (r = -0.362; p < 0.05) in malaria and HIV co-infected group. The CD4⁺ T-cell count, WBC count, PCV were not significantly different between the Malaria-HIV co-infection group and HIV infection group.

Interpretation & conclusion: In the present study serum iron and albumin concentrations were the most sensitive indicators that showed the contributory impact of malaria infection on biochemical index in HIV co-infected subjects. The findings suggest that at the defined stage of HIV infection in the present study, malaria co-infection may moderate the impact of HIV infection on iron metabolism and hepatic synthesis of albumin.

Key words Biochemical-index – HIV– malaria co-infection

Introduction

Malaria is endemic in Nigeria with stable transmission over the year and high prevalence of asymptomatic malaria has been reported in pregnant women in

this region^{1,2}. The spread of human immunodeficiency virus (HIV) is also high within this area³. Thus, we have shown evidence of malaria-HIV co-infection among the populace with prevalence of malaria parasitaemia being higher amongst symptomatic

HIV infected subjects³. The advent of HIV infection in the sub-Saharan Africa has also led to high prevalence of malaria infection in HIV subjects in areas with stable transmission^{3,4} and unstable transmission^{5,6}. Presentation of severe malaria has been observed in HIV subjects with CD4⁺ T-cell count less than 200×10^6 cells/L⁷. The risk was shown to be higher in non-immune malaria subjects who usually present with a high parasite and WBC counts⁷. In our recent observation³, we reported that prevalence of malaria infection is tripled amongst symptomatic HIV-infected subjects, however, the prevalence amongst the asymptomatic HIV-infected subjects was similar to that expressed by the apparently healthy HIV seronegative individuals within the same area. Hence, the present study was designed to determine the possible contributory impact of malaria parasitaemia on some biochemical markers in subjects with HIV co-infection.

Material & Methods

Subjects participated in the study were recruited at the Voluntary Counseling and Testing (VCT) Unit, Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria. At the VCT, individuals are counseled on HIV infection, health implications and management of a positive HIV test. While recruiting at the VCT, individuals were screened for HIV infection, *Plasmodium falciparum* antigen and malaria parasite detection. The participants with positive HIV test were further staged into asymptomatic (stage-1) and symptomatic (stage-2) using the World Health Organization (WHO) guidelines on HIV staging. Stage-3 and stage-4 individuals were excluded in the present study. The recruited participants were not on anti-retroviral therapy. Based on the above screening result, the participants were subsequently divided into following groups.

Malaria and HIV co-infected group: Consists of 45 participants aged 36 ± 10 years (male = 15, females = 30) with both malaria and HIV co-infection and 28 of

these participants presented with '*symptomatic HIV co-infection*' while the remaining 17 participants presented with '*asymptomatic HIV co-infection*'.

HIV infected group: Consists of 57 participants aged 37 ± 10 years (male = 20, females = 37) with HIV infection without concurrent malaria infection and 30 of these participants presented with '*symptomatic HIV infection*' while the remaining 27 participants presented with '*asymptomatic HIV infection*'.

Blood samples collected were also used for malaria parasite density determination; CD4⁺ T-cell count, packed cell volume (PCV), white blood cell count (WBC), serum iron and albumin levels. The study was carried out between the month of May and August, a predominantly rainy season in Nigeria. The participants gave informed consent while the NAUTH ethical committee approved the study design.

Method for HIV screening was as previously described³. Method for *P. falciparum* histidine rich protein seroreactivity and parasite density was as previously described^{2,3}. Bromocresol Green (BCG) method was used to determine serum albumin concentration.

Determination of the packed cell volume: Anti-coagulated (EDTA) blood sample from each participant was aspirated into micro-haematocrit capillary tubes. One end of the capillary tube was subsequently sealed and placed in the micro-haematocrit centrifuge. A constant packing of the red blood cells was achieved with a centrifugation speed of $1200 \times g$ for 3 min. The PCV was measured and reported as a ratio of the whole blood volume in litre/litre.

Determination of CD4⁺ T-cells count by Cyflow SL Green: About 50 μ l of whole blood in EDTA anti-coagulant was dispensed into a partec test tube and 10 ml of CD4 PE antibody was added. The reaction mixture was mixed and incubated in the dark for

10–15 min. After the incubation, 800 µl of the already prepared diluted buffer was added to each reaction tube and vortexed. The partec tubes containing these reactions were plugged in position in the Cyflow SL Green (Partec, Germany), which has already been connected to flow max software, CD4 count template data file and CD4 count instrument. The test was run on the Cyflow for 90 sec. The results were displayed as histogram and printed. The CD4⁺ T-cell count was read off the histogram correcting for the dilution factor.

Serum iron determination by colorimetric method: The procedure was as described by the manufacturer of the kit (Chromatest, Barcelona, Spain). In brief, 200 µl of the sample from each participant was put in appropriately labeled test tube containing 1.0 ml of working solution [containing one part of chromogen solution (ferrozine 40 mmol/L and sodium acetate 400 mmol/L) and four parts of buffer/reductant solution (Guanidine chloride 1.0 mol/L; hydroxylamine 0.6 mol/L; acetate buffer 400 mmol/L pH 4.0; teepol mixture)]. Similar procedure was used for the reagent blank and standard test tubes, except that the sample blank was prepared with addition of 1.0 ml of the buffer/reductant solution. The reactions were incubated at room temperature for 5 min, after which the absorbance of the sample blank was read at 560 nm against distilled water while the absorbance of the sample tests and standards were read at 560 nm against reagent blank. Subsequently, the concentration of serum iron was calculated.

Determination of WBC counts using tuerk's solution: Into appropriately labeled test tubes containing 950 µl of tuerk's solution (2% glacial acetic acid and 50 µl of gentian violet), 50 µl of EDTA anti-coagulated blood sample of each participant was added respectively. The solution was allowed to stand for 5 min and through capillary action was loaded unto the new improved nurber chamber. The population of WBC in the four corner cells was read under the microscope using x10 objective lens. The amount of

WBC was then calculated for each participant adjusting for the dilution.

Statistical analysis: The variables were expressed as mean (\pm SD). The independent student *t*-test was used to assess significant mean differences. The Spearman's correlation coefficient was used to assess the level of association between two variables. Significant level were considered at <0.05 and <0.01 .

Results

The mean serum iron concentration in malaria and HIV co-infected group (107.5 ± 53.5) was significantly lower than that of in HIV infected group (151.9 ± 79.3) without concurrent malaria infection ($p < 0.05$). Similarly, the mean serum albumin concentration in malaria and HIV co-infected group (42 ± 9.6) was significantly higher than the HIV infected group (38.4 ± 7.5) without concurrent malaria infection ($p < 0.05$). On the contrary, WBC count, CD4⁺ T-cell count and PCV were similar in both groups ($p > 0.1$ in each case). The blood *P. falciparum* parasite density in the malaria and HIV co-infected group was $667 \times 10^3 \pm 463 \times 10^3$ (Table 1).

The mean serum iron concentration in participants with malaria and asymptomatic HIV co-infection was significantly lower compared to that of participants with asymptomatic HIV infection without concurrent malaria infection ($p < 0.01$). Similarly, the mean serum albumin concentration in participants with malaria and asymptomatic HIV co-infection was significantly higher than that of participants with asymptomatic HIV infection without concurrent malaria infection ($p < 0.05$). Other parameters were similar between both groups (Table 1).

When comparisons were made between the malaria and symptomatic HIV co-infected participants and symptomatic HIV infected participants without concurrent malaria infection, no significant mean difference was observed between both groups (Table 1).

Table 1. Mean (\pm SD) blood concentrations of WBC, PCV, CD4⁺ T-cells, *P. falciparum* density and serum concentrations of iron and albumin in study subjects

| Parameters | HIV-infected | Malaria-HIV co-infected | p-value |
|--|------------------|-------------------------|---------|
| <i>Symptomatic & asymptomatic</i> | <i>n = 57</i> | <i>n = 42</i> | |
| WBC ($\times 10^6/L$) | 4.16 \pm 1.24 | 3.93 \pm 1.25 | >0.1 |
| PCV (L/L) | 0.32 \pm 0.04 | 0.32 \pm 0.08 | >0.1 |
| CD4 ⁺ T-cells (/mm ³) | 285 \pm 124 | 292 \pm 149 | >0.1 |
| Serum iron ($\mu\text{g/dL}$) | 151.8 \pm 79.3 | 107.5 \pm 53.5 | <0.05 |
| <i>Pf</i> density ($\times 10^3/L$) | – | 667 \pm 463 | nc |
| Serum albumin (g/L) | 38.4 \pm 7.5 | 42 \pm 9.6 | <0.05 |
| <i>Asymptomatic</i> | <i>(n = 26)</i> | <i>(n = 18)</i> | |
| WBC ($\times 10^6/L$) | 4.18 \pm 1.23 | 4.02 \pm 1.19 | >0.1 |
| PCV (L/L) | 0.34 \pm 0.04 | 0.35 \pm 0.08 | >0.1 |
| CD4 ⁺ T-cells (/mm ³) | 319 \pm 81 | 326 \pm 157 | >0.1 |
| Serum iron ($\mu\text{g/dL}$) | 133.4 \pm 53.3 | 81.2 \pm 36.7 | <0.01 |
| <i>Pf</i> density ($\times 10^3/L$) | – | 803 \pm 526 | nc |
| Serum albumin (g/L) | 37.2 \pm 5.6 | 43.1 \pm 6.5 | <0.05 |
| <i>Symptomatic</i> | <i>(n = 30)</i> | <i>(n = 28)</i> | |
| WBC ($\times 10^6/L$) | 4.14 \pm 1.27 | 3.89 \pm 1.30 | >0.1 |
| PCV (L/L) | 0.31 \pm 0.04 | 0.30 \pm 0.07 | >0.1 |
| CD4 ⁺ T-cells (/mm ³) | 257 \pm 148 | 266 \pm 140 | >0.1 |
| Serum iron ($\mu\text{g/dL}$) | 171.7 \pm 98.6 | 127.3 \pm 56.4 | >0.1 |
| <i>Pf</i> density ($\times 10^3/L$) | – | 604 \pm 440 | nc |
| Serum albumin (g/L) | 39.3 \pm 8.6 | 41.5 \pm 10.9 | >0.1 |

nc = Not compared.

Furthermore, sex distribution of the evaluated parameters showed no significant differences irrespective of the group.

A positive association was observed between age and serum iron concentration in malaria and HIV co-infected group ($r = 0.580$; $p < 0.05$). However, negative associations were observed between PCV and serum iron ($r = -0.388$; $p < 0.05$) and between CD4⁺ T-cells and serum iron concentration ($r = -0.362$; $p < 0.05$) in malaria and HIV co-infected group. Positive associations were observed between CD4⁺ T-cell count and serum iron ($r = 0.487$; $p < 0.05$); and between CD4⁺ T-cells and serum albumin ($r = 0.301$; $p < 0.01$)

in HIV infected group without concurrent malaria infection.

Discussion

The present study was designed to assess the contributory impact of malaria infection in subjects with HIV co-infection. The serum iron concentration was the most sensitive indicator of possible impact of malaria infection in subjects with HIV co-infection. The study clearly revealed that serum iron was reduced in malaria and HIV co-infected group compared with HIV infected group without concurrent malaria infection. The reduced serum iron concentration was also particularly true amongst the malaria and asymptomatic HIV co-infected participants but not truly in malaria and symptomatic HIV co-infected participants.

The positive association observed between serum iron and age in malaria and HIV co-infected group, might be an indication that the reduced serum iron might be more pronounced with age in such individuals with co-infection. This finding is with respect to a malaria endemic area with stable transmission throughout the year.

The negative association observed between serum iron and PCV and on the other hand between serum iron and CD4⁺ T-cell count, in malaria and HIV co-infected group calls for critical analysis and interpretation. This is because of the possible implications and impact of free iron on health. CD4⁺ T-cell count has been used generally to define stability of HIV infected subjects with counts of below 200 regarded as indication of poor prognosis. Hence, the result of negative association between serum iron concentration and CD4⁺ T-cell count could well deduce that reduced serum iron in malaria-HIV co-infection is indicative of good prognosis.

In HIV infection, serum ferritin level has been shown to be positively associated with viral load⁸. In a study

elsewhere amongst asymptomatic malaria subjects serum iron level has been shown to be similar to that observed in uninfected control subjects⁹. In another study amongst apparently healthy individuals in Nigeria with malaria parasitaemia, serum iron level was found to be similar in both the parasitaemic and aparasitaemic apparently healthy individuals¹⁰. A possible role has been defined for iron in HIV infection⁸ and serum ferritin has been reported to be lower in HIV infected subjects compared with malaria parasitaemic subjects¹¹.

Thus, the finding in the present study and those reported above could suggest that the possible source of increased serum iron in HIV infection may be depletion of iron store. But such depletion of iron store and release into circulation may be disrupted in cases of malaria co-infection. This process of depletion of iron store may be reversed in cases of concurrent malaria infection, thus favouring reduced iron in transport with consequent increase in storage. However, it may be necessary to check the total iron binding capacity in these individuals as it may help to further understand the metabolic mechanism of iron. This is because when the malaria-HIV co-infected group was further considered based on HIV staging the serum iron remained reduced in asymptomatic but not in symptomatic HIV co-infected participants.

Studies elsewhere have shown occurrence of severe malaria in non-immune subjects with HIV co-infection with CD4⁺ T-cell count of below 200 cells⁵. However, they were unable to observe severe malaria in subjects with HIV co-infection from endemic area⁵. In their report, increase in WBC and parasite counts were only observed in non-immune subjects with HIV co-infection. In another report from Malawi, CD4 cell count was found to be moderately but inconsistently associated with malaria parasitaemia¹².

In the present study, the mean CD4⁺ T-cell count was neither below 200 cells nor was there any significant

difference in means among subjects with malaria-HIV co-infection and HIV infection alone. This may be an indication that malaria infection in these subjects did not have any contributory suppressive impact on the CD4⁺ T-cells count or that T-cell function is still fairly well. Similarly, we have observed that neutrophil ingestion of nitroblue tetrazolium was impaired in both HIV-malaria co-infection and HIV infection alone, however, no contributory impairment due to malaria was noticed¹³.

A striking observation is the significant increase in serum albumin in malaria and HIV co-infected group which could be an indication that malaria infection in endemic area may interact to reverse the progressive distortion of synthesis of albumin by the liver during HIV infection. This observation may not be attributed to dehydration considering the PCV observed in same subjects. This calls for further investigation because serum albumin has a strong prognostic value in HIV infection^{14,15}.

In the present study, serum iron and albumin concentrations were the most sensitive indicators that showed the contributory impact of malaria infection on biochemical index in HIV co-infected subjects. The findings suggest that at the defined stage of HIV infection in the present study, malaria co-infection may moderate the impact of HIV infection on iron metabolism and hepatic synthesis of albumin. It could be that for the CD4⁺ T-cell count observed, certain level of immune regulation may still exist in these subjects and ability to keep in check the level of parasitaemia. Thus, malaria infection in HIV infected subjects in malaria endemic area may not be implicated as contributing adverse effect on some biochemical markers in HIV co-infected subjects.

References

1. Onyenekwe CC, Meludu SC, Dioka CE, Salimonu LS. Prevalence of asymptomatic malaria parasitaemia amongst pregnant women. *Indian J Malariol* 2002; 39: 60–5.

2. Onyenekwe CC, Arinola OG, Salimonu LS. Detection of *Plasmodium falciparum*-IgG and incidence of asymptomatic malaria in pregnant women in Nigeria. *Indian J Malariol* 2002; 39: 39–42.
3. Onyenekwe CC, Ukibe N, Meludu SC, Ilika A, Aboh N, Ofiaeli N, Ezaeni M, Onochie A. Prevalence of malaria as co-infection in HIV infected individuals in a malaria endemic area of Southeastern Nigeria. *J Vector Borne Dis* 2007; 44: 250–4.
4. Abu-Raddad LJ, Patnaik P, Kublin JG. Dual infection with HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. *Science* 2007, 315(5812): 598.
5. Grimwade K, French N, Mbatha DD, Zungu DD, Dedicoat M, Gilks CF. Childhood malaria in a region of unstable transmission and high human immunodeficiency virus prevalence. *Pediatr Infect Dis J* 2003; 22(12): 1057–63.
6. Grimwade K, French N, Mbatha DD, Zungu DD, Dedicoat M, Gilks CF. HIV infection as a co-factor for severe falciparum malaria in adults living in a region of unstable malaria transmission in South Africa. *AIDS* 2004; 18(3): 547–54.
7. Cohen C, Karstaedt A, Frean J, Thomas J, Govender N, Prentice E, Dini L, Galpin J, Crewe-Brown H. Increased prevalence of severe malaria in HIV-infected adults in South Africa. *Clin Infect Dis* 2005; 41(11): 1631–7.
8. Friis H, Gomo L, Nyazema N, Ndhlovu P, Krarup H, Madsen PH, Michaelsen KF. Iron haptoglobin phenotype and HIV-1 viral load: a cross-sectional study among pregnant Zimbabwean women. *J Acquir Immune Defic Syndr* 2003; 33(18): 74–81.
9. Das BS, Thumhan DI, Das DB. Influence of malaria on marker of iron status in children: implications for interpreting iron status in malaria endemic communities. *Br J Nutr* 1997; 78(5): 751–60.
10. Odunukwe NN, Salako LA, Okany C, Ibrahim MM. Serum ferritin and other haematological measurement in apparently healthy adults with malaria parasitaemia in Lagos, Nigeria. *Trop Med Int Health* 2000; 5(8): 582–6.
11. Friis H, Gomo L, Koestel P, Ndhlovu P, Nyazema N, Krarup H, Michaelsen KF. HIV and other predictors of serum folate, serum ferritin and haemoglobin in pregnancy: a cross-sectional study in Zimbabwe. *Am J Clin Nutr* 2001; 73(5): 1066–73.
12. Patnaik P, Jere CS, Miller WC, Hoffman IF, Wirima J, Pendame R, Meshnick SR, Taylor TE, Molyneux ME, Kublin JG. Effect of HIV-1 serostatus, HIV-1 RNA concentration, and CD4 cell count on the incidence of malaria infection in a cohort of adults in rural Malawi. *J Infect Dis* 2005; 172: 984–91.
13. Onyenekwe CC, Ukibe NR, Meludu SC, Ifeanyi CM, Igwegbe AO, Ezeani M, Ezeugwunne I, Ofiaeli N, Onochie A, Ilika A, Abor N. Neutrophil ingestion rate of nitroblue tetrazolium in subjects with malaria-HIV co-morbidity. *Trop J Med Res* 2008; 12(1): 1–5.
14. Joseph G, Feldman PH, David N. Serum albumin as a predictor of survival in HIV-infected women in the women interagency HIV study. *AIDS* 2000; 14(7): 863–70.
15. Olawumi HO, Olatunji PO. The value of serum albumin in pre-treatment assessment and monitoring of therapy in HIV/AIDS patients. *HIV Med* 2006; 7: 351–5.

Corresponding author: Dr Charles C. Onyenekwe, Department of Chemical Pathology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Anambra State, Nigeria.
E-mail: charleschinedum2002@yahoo.com.

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