Iron Studies of HIV-Infected Subjects in Calabar- A Nigerian Perspective

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Abstract
Investigating anaemia through screening tests could be insufficient in managing chronic infections. This study investigated the involvement of iron in the anaemia of human immunodeficiency virus infection. Participants were aged 19 to 50 years, comprising 76 infected subjects and 53 controls. The CD4+ count and haemoglobin concentration were determined by automation. Serum iron and total iron binding capacity were determined colorimetrically, while serum ferritin and interleukin-6 were analyzed by enzyme linked immunosorbent assay. Percentage of treatment-naïve subjects (61%) exceeded that of subjects on treatment (39%). Generally, 12%, 16% and 8% of the infected subjects had lower serum iron, total iron binding capacity and ferritin values respectively while 50%, 51% and 35% had values higher than the reference values respectively. However 38%, 33% and 57% of the infected subjects had their values within the reference limits. Approximately 45% of infected subjects had lower haemoglobin concentration, while 55% had values within range. The iron parameters of treatment naïve infected subjects did not significantly (P > 0.05) change when compared with those on treatment but significantly changed (P < 0.5) when compared with the control group. Degree of immunosuppression adjudged by the CD4+ count was 31.5% for severe 48.7% advanced, 14.5% mild and only 5.3% normal. There are greater percentages of treatment naïve subjects as well as severe immunosuppression in this locality. Approximately 45% anaemia prevalence was recorded in the studied infected population but the anaemia may not be a reflection of iron deficiency.

Keywords: Anaemia, Iron Studies, Human Immunodeficiency Virus.

Introduction
Since the discovery of human immunodeficiency virus (HIV), tremendous advances in the management of HIV infection have been recorded. Therapeutic approaches with proper adherence aim mainly at achieving suppression of viral load which by extension should minimize both morbidity and mortality [1,2]. This again is largely dependent on early diagnosis and intervention [3]. Among infected persons, anaemia has remained a

Euphoria C. Akwiwu et al JMSCR Volume 05 Issue 03 March  
Page 19572
frequent complication \cite{4,5} and determinant of survival \cite{6}. This burden together with reports of widespread iron deficiency in developing countries has probably compounded the combat against HIV infection in such regions. Clinical assessment has reportedly dominated the diagnosis of anaemia especially in Africa. Although laboratory testing has been prioritized in the determination of anaemia in the management of HIV, investigation of anaemia has remained at a general screening level, while iron deficiency has been assumed to be the most common cause of anaemia \cite{7}. The more specific assays including iron studies, capable of highlighting underlying nature of anaemia are not usually carried out routinely. A possible consequence of this limitation is the lack of insight towards proper management of infected subjects; with attending increased morbidity and mortality rates. This study sought to investigate the involvement of iron in the anaemia of HIV-infection in our locality.

**Methods**

This research work was carried out in University of Calabar Teaching Hospital Calabar, Nigeria. Seventy-six (76) male and female subjects serologically diagnosed for HIV infection at the centre whose ages were within 19 and 50 years and control subjects drawn from the populace with the same age range and are sero-negative for HIV were enrolled. Ethical approval was obtained from the University of Calabar Teaching Hospital Medical Ethical Committee, while informed consent was given by each participant. Five milliliters of venous blood was collected aseptically from each subject out of which 3mls was dispensed into a plain tube for serum harvesting and the remaining 2mls dispensed into dipotassium ethylene diamine tetra-acetic acid (EDTA K\textsubscript{2}) bottle for CD4 T-cell count and haemoglobin concentration (Hb). Immunochromatographic method was employed for HIV screening using Determine HIV 1/2 (Alere Medical Company Ltd, Matsudoshi, Japan, Lot No: 42532k100) and Chembio HIV 1/2 (Stat-Pak Assay. L ot No: 1106151A). The CD\textsubscript{4}T-cell count was conducted using Partec cyflow cytometer, while haemoglobin concentration (Hb) was carried out using Sysmex KX-21N from Sysmex Corporation, Japan. Serum iron (SI) and total iron binding capacity (TIBC) were determined calorimetrically with test kits from TECO diagnostics, USA. Serum ferritin (SF) and IL-6 were analyzed using enzyme linked immunosorbent assay test kits from Assaypro, USA. SPSS 19.0 was used for the statistical analyses of data. A two tailed P-value of $\leq 0.05$ was considered indicative of a statistically significant difference.

**Results**

This case-control research examined the iron parameters and interleukin 6 levels of HIV Sero-positive subjects attending University of Calabar Teaching Hospital, Calabar. The demographic characteristics of the studied population revealed more participation by females compared to males. However, the percentage of treatment-naïve subjects (61%) exceeded that of the patients on ART (39%) (Table 1). Twelve percent (9/76), 38% (29/76) and 50% (38/76) of HIV infected subjects had values below, within and above the reference range respectively for serum iron. TIBC values showed 16% (12/76), 33% (25/76) and 51% (39/76) as lower, within range and higher values respectively. For serum ferritin, 8% (6/76) had lower values, 57% (43/76) had values within range, while 35% (27/76) had higher values. The pattern for Hb concentration shows that 45% (34/76) had lower values whereas 55% (42/76) had values within range (Fig 1).

The iron parameters together with the CD4\textsuperscript{*} count and IL 6 were considered for HIV subjects not yet placed on treatment (treatment-naïve), the ones undergoing treatment as at the time of the study and control group as shown in Fig 2. Based on these categories respectively, SI ($\mu$g/dL) values were recorded as 117.59±109.82 for treatment-naive group and did not significantly change when
compared with those on ART 228.40±181.33. However, a significant change (P<0.05) was observed when comparison was made between the control group 80.61 (61.00) and those on ART as well as those who are treatment-naïve. Similar pattern was recorded for TIBC (ug/dL) naïve 472.80±207.09 those on treatment 456.96±254.76 and control group 425.48±305.45, interleukin-6 (ng/mL) 10.16±4.34 naïve, those on treatment 6.08±2.24 and control 2.18±1.16. The SF (ng/mL) values were 232.66±139.99, 220.54±143.00 and 112.57±101.49 respectively for naïve, treatment and control group. Mean values for Hb (g/L) were 119.2±14.2, 123.6±16.5 and 135.7±15.9 respectively for naïve, treatment and control. On the other hand CD4\(^+\) count (cells/mL) showed significant difference (P<0.05) when subjects on ART (379.45±101.42) was compared with those not on ART (183.60±62.23) as well as with the control group (756.59±168.29). Furthermore CD4\(^+\) count was used to grade the degree of immunosuppression into severe (<200 cells/mL), advanced (200-349 cells/mL), mild (350-499 cells/mL) and normal (≥500 cells/mL). The percentages of subjects that fell into these grades were 31.5%, 48.7%, 14.5% and 5.3% respectively as shown in table 2. However, it was observed that 24 subjects with severe immunosuppression as well as 21 of the advanced cases were yet to be placed on ART (Table 2). The remaining 16 persons of the advanced immunosuppressed subjects as well as all those with mild immunosuppression 11 and normal cases 4 were undergoing ART (Table 2).

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**Table1.** Demographic Characteristics of the Studied Population

| Participants            | HIV Sero-positive Patients (Test Subjects) | HIV Sero-negative Individuals (Control Subjects) |
|-------------------------|-------------------------------------------|-----------------------------------------------|
|                         | HIV Sero-positive Patients (Test Subjects) | HIV Sero-negative Individuals (Control Subjects) |
|                         | Females (58%)                            | 31 (58%)                                      |
|                         | 44 (58%)                                 | 31 (58%)                                      |
|                         | Males (42%)                              | 22 (42%)                                      |
|                         | 32 (42%)                                 | 22 (42%)                                      |
| Total                   | 76 (100%)                                | 53 (100%)                                     |
| Treatment-naïve         | 46 (61%)                                 | -                                             |
| Treatment-experienced   | 30 (39%)                                 | -                                             |
| Total                   | 76 (100%)                                | -                                             |

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**Fig.1.** Percentage distribution of HIV infected subjects across the reference ranges of the iron parameters.
Fig. 2. Expression of measured parameters of infected patents yet to be placed on treatment, those undergoing ART and control subjects

Table 2. Degree of Immunosupression

| CD4+ GRADING | SEVERE | ADVANCED | MILD | NORMAL |
|--------------|--------|----------|------|--------|
| <200         | n=24   | 200-349  | 350-499 | ≥500   |
|              | 31.5%  | 48.7%    | 14.5% | 5.3%   |

| Percentage degree of immunosupression | 31.5% | 48.7%, 14.5% | 5.3% |
| Number of Treatment-naïve | 24 | 21 (56.8%) | - | - |
| Number on ART | - | 16 (43.2%) | 11 | 4 |

**Discussion**

Highly active antiretroviral therapy (HAART) is the widely employed approach in the management of HIV infection and much of the current outcome of disease course has been linked to its advent [8]. International assistance has tremendously aided the provision of medical care to infected patients from developing countries including Nigeria. However, anaemia in association with HIV infection has remained a source of concern especially as it persists among patients on therapy. This occurrence is thought to mediate HIV disease progression [9,10] and thus has necessitated further investigations targeted at understanding the underlying factors to anaemia including iron studies.

The study showed 61% of the subjects yet to be placed on ART arising partly from late presentation at hospital, and 58% female participation probably owing to the mandatory antenatal screening. Overall, iron parameters were observed to be mainly within or above reference ranges for the SI, TIBC and SF. Relatively adequate iron stores observed in this study indicates a lesser involvement of iron deficiency in the anaemia of HIV; a pattern that has been recorded in a similar setting [11]. However this issue of imbalance in iron metabolism has been considered as a mediator of disease progression among the HIV population [12]. Commencement of ART slightly addressed this imbalance in iron metabolism though not significantly. This trend...
was also observed for the serum IL-6; probably reflecting gradual immunologic improvement as previously observed \[^{13}\]. Although majority of these patients presented with either advanced or severe immunosuppression on the basis of the CD4\(^+\) count, there was appreciable improvement (p<0.05) of the same parameter among patients on ART. Of the 37 (48.7\%) that had advanced stage, more than half 21 (56.8\%) were yet to enroll for ART, while the remaining 16 (43.2\%) were on an upward recovery slope after commencing therapy. Unfortunately again, all the 24 (31.5\%) with severe degree of immunosuppression were not yet on therapy as at the time of the study. The cutoff value of 350 cells/mL for CD4\(^+\) count as the criterion for commencing ART has since been reviewed to an upward value of 500 cells/mL by WHO since 2013 \[^{14}\] but the finding from this work has shown that the criterion may not have been implemented in this center as 45 of 76 persons (59\%) studied had their CD4\(^+\) count below 350 and are yet not on ART. The present study has observed higher degrees of immunosuppression among HIV infected persons in our locality, a delicate situation that is capable of compromising the health of affected individuals. Our observation for the commencement of care for these infected persons is often hampered by late presentation of subjects to hospitals and consequently late diagnosis. This trend that has trailed the management of HIV as previously reported \[^{15}\] is still plaguing. Therefore efforts in the use of CD4\(^+\) count in the staging, treatment and monitoring of HIV/AIDS should be maintained and sustained, while early and immediate commencement of ART upon diagnosis as directed by WHO should be implemented and adhered to in the treatment, monitoring and management of HIV/AIDS. However, factors that militate against early presentation of subjects to hospital including issues that border on stigmatization should be investigated.

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