Anaemia and Pro-inflammatory Cytokines in Human Immunodeficiency Virus Infection

Chiamaka Loveth Enumah, Chijioke Adonye Nwauche, Anthonia Okerengwo

Department of Haematology, Blood transfusion and Immunology, University of Port Harcourt, Port Harcourt, Nigeria

Email address: cifiorah@yahoo.com (C. L. Enumah)

Abstract: Anaemia is often seen in HIV infected individuals and cytokines imbalance have been reported to cause haematopoietic stem cells suppression in the bone marrow. This case control study was carried out to determine any possible correlation between haemoglobin and pro-inflammatory cytokines in the development of anaemia in people living with HIV. 54 HIV infected and 34 apparently healthy HIV uninfected individuals were enrolled in this study. Full blood count and levels of three pro-inflammatory cytokines [(IL- α (interleukin-1 alpha), TNF-α (tumour necrosis factor- alpha) and IFN- γ (interferon- gamma)] were determined using MindrayBC- 6800 automated Haematology analyser and Enzyme linked Immunosorbent Assay (ELISA) kits (Aviva system Biology, San Diego, CA, USA) respectively. The results obtained showed that prevalence of anaemia was 40.7% in HIV infected subjects, with higher prevalence in females (54.5%) than in males (19.0%). The difference in the mean concentrations of haemoglobin, red blood cell, packed cell volume, IL- α and TNF-α was statistically significant (p<0.05) while white blood cell counts and IFN- γ were not statistically significant (p>0.05) when compared to the control group. There were weak correlations between haematological parameters and pro-inflammatory cytokines in the HIV positive group. HIV subjects were later divided into treatment groups: those on highly active antiretroviral therapy (HAART) - Zidovudine-Lamivudine- Nevirapine (ZLN) combination, Tenofovir-Lamivudine- Efavirenz (TLE) combination and those that are not on HAART- treatment naïve patients (TNP). There were statistically significant differences in the mean concentrations of red blood cell and white blood cell across the TLE and TNP groups (p<0.05). There were positive correlations between IFN-γ and HB in TLE group but negative correlations in other two groups. Also, there were positive correlations between IFN-γ and WBC in ZLN group but negative correlations in other two groups. This study suggests that the dysregulated pro-inflammatory cytokine levels caused by HIV itself played a role in causing anaemia in HIV infections.

Keywords: Haemoglobin, Interleukin-1 alpha, Tumour Necrosis Factor- Alpha, Interferon- Gamma, Human Immunodeficiency Virus, Enzyme Linked Immunosorbent Assay, Highly Active Antiretroviral Therapy

1. Introduction

The human immunodeficiency virus (HIV) is a causative agent for acquired immunodeficiency syndrome (AIDS). Its effect on the body is rapid destruction of the body’s immune system which can lead to opportunistic infections, immunological and haematological complications [1]. According to World Health Organisation, anaemia is considered to be when haemoglobin, packed cell volume and red cell count levels are below the normal reference ranges.

In males, anaemia is diagnosed when haemoglobin is less than 13g/dl, packed cell volume is less than 39% and red blood cell count is less than 4.5 million while in females, anaemia occur when haemoglobin is less than 12g/dl, packed cell volume is less than 36% and red cell count is less than 3.9 million [2].

Cytokines are soluble glycoproteins released into the body to control immune responses, development of blood cells and wound healing [3]. Cytokines are classified into monokines, interferons, interleukins, lymphokines and tumor necrosis factor, depending on their functions and producing cells [3].
Depending on their inflammatory actions, cytokines are also categorized into:

i. Pro-inflammatory cytokines- These are known to promote inflammation. They include: interleukin-1 (IL-1), IL-2, interferon- gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α), IL-6, IL-12 and IL-8.

ii. Anti-inflammatory cytokines known to suppress the activity of pro-inflammatory cytokines include: IL-35, IL-10 and IL-4.

2. Background of Study

Anaemia is the most leading haematologic anomaly in HIV infection and is one of the predictors of HIV progression to AIDS [4]. Defective haematopoiesis, clotting disorders and reduction of immune cells have been associated with HIV infection [5]. Adverse events occurring as a result of HAART, the virus itself, opportunistic infections or cancer may be attributed to these impairments in HIV infection [5].

In a study carried out in University of Port Harcourt Teaching Hospital, Nigeria, it was reported that suppression of the bone marrow and depletion of immune cells by HIV itself may have contributed to anaemia observed in the HIV infected persons [6]. Also, a comparative cross sectional study in Ghana, postulated that anaemia in HIV infection was as a result of the virus itself more than HAART related [7]. Anaemia has been found in both naïve and HAART HIV patients suggesting that HIV may have caused defective physiologic activities thus impaired haematopoiesis [8]. A HAART known as Zidovudine has been reported to cause anaemia in people living with HIV by causing intoxication of the bone marrow and marked decrease in haemoglobin concentration [9-11]. However, some investigations carried out in Nigeria and Tanzania suggested that highly active antiretroviral therapy (HAART) initiation may improve haematologic parameters in HIV infection [12, 13].

Some investigators have demonstrated that cytokines in the plasma of HIV-infected patients may prevent or reduce the activities of haematopoiesis, or suppress bone marrow progenitor cells. This may lead to anaemia and other haematologic anomalies in HIV infection [14, 15]. Also, a model has been developed in which the pathophysiologic processes producing the anaemia in chronic diseases like HIV may be linked to the actions of pro-inflammatory cytokines (IL-1, TNF-α, and IFN-γ) that mediate the immune responses [16]. Another study suggested that HIV may deplete a number of cytokine that play a major role in red blood cell production [17]. It has been further postulated that virus proliferation might reduce the normal body physiologic response to anaemia and regulatory cytokines that maintain normal haematopoiesis [8]. Furthermore, onset of HIV infection may bring about imbalance in cytokine secretion between the viral inducers and viral suppressors [18, 19]. Some cytokines have been demonstrated to regulate HIV infection in vitro by secretion of high levels of IL-6, IL-10, IL-4 and reduced secretion of IL-12, IFN-gamma and IL-2 [20]. It has also been suggested that an imbalance in production of cytokine is responsible for the impaired immune cells and characteristic progression to AIDS or even death [21].

Understanding the mechanisms mediating anaemia and pro-inflammatory cytokines processes that occur in HIV infection will contribute to improving therapeutic options and disease management.

3. Materials and Methods

3.1. Ethical Approval

Ethical approval was obtained from the Ethical committee of University of Port Harcourt Teaching Hospital and University of Port Harcourt, Rivers State, Nigeria before the commencement of the study. Verbal informed consent was also obtained from all patients recruited for the study.

3.2. Study Design

This was a case control study carried out in HIV clinic and Department of Haematology and Blood transfusion both in University of the Port Harcourt Teaching Hospital, Rivers State, Nigeria.

3.3. Study Population

The study population consisted of 54 HIV infected (21 males and 33 females) and 34 apparently healthy HIV non-infected (29 males and 5 females) individuals. The HIV infected subjects are those on HAART and HAART treatment naive.

3.4. Inclusion and Exclusion Criteria

People living with HIV/AIDS (PLWHA) and apparently healthy HIV- negative persons who met the criteria for blood donation were eligible for enrolment into the study. All subjects were between 18-50 years. Neonates, children, adults older than fifty (50) years, pregnant women, sickle cell anaemic patients and females menstruating at the time of samples collection were excluded.

3.5. Specimen Collection and Preservation

5mls of venous blood were aspirated from each patient using aseptic procedures and dispensed into EDTA bottles. Full blood count (FBC) was carried out the same day of sample collection for each of the samples. The blood was centrifuged, plasma separated and dispensed into a plain tube. The plasma was stored at -20°C until cytokine assay was carried out.

3.6. Analysis of Full Blood Count and Cytokine Concentration

Full blood count was done using MindRay BC – 6800 automated haematology analyzer. Cytokine levels were estimated using Enzyme Linked Immunosorbent Assay (ELISA) kits (Avivabio System, San Diego, CA, USA).
3.7. Calculation of Results

The readings for each standard, control and samples were taken and used to generate a standard curve, by reducing the data using Graphpad prism software package version 6.0. Cytokine concentrations were determined with corresponding optical densities on the standard curves.

3.8. Statistical Data Analysis

The chi-square ($\chi^2$) statistical tool was used to test for difference in categorical variables (gender and age distribution) of the subjects. Analysis of Variance (ANOVA) for difference among group means was used to analyse the difference in haematological parameters and cytokine concentrations across the different groups. Pearson’s correlation was used to compute the differences between cytokines values and haematological parameters. All tests were carried out using the Graphpad Prism version 6 Software at a 95% confidence interval and a p-value ≤ 0.05 was considered significant.

4. Results

Table 1. Distribution of study groups according to gender and anaemia status.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Anaemic HIV positive group (n, %)</th>
<th>Non-anaemic HIV positive group (n, %)</th>
<th>Control (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>4 (19.1)</td>
<td>17 (53.1)</td>
<td>29 (85.3)</td>
</tr>
<tr>
<td>Female</td>
<td>18 (81.8)</td>
<td>15 (46.9)</td>
<td>5 (14.7)</td>
</tr>
<tr>
<td>Total</td>
<td>22 (100.0)</td>
<td>32 (100.0)</td>
<td>34 (100.0)</td>
</tr>
</tbody>
</table>

The prevalence of anaemia in female HIV positive subjects (54.5%) was significantly higher than in male HIV positive subjects (19.0%). The prevalence of anaemia in the combined HIV positive group was 40.7%.

Table 2. Age distribution of HIV positive and negative (control) groups.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Anaemic HIV positive group (n, %)</th>
<th>Non-anaemic HIV positive group (n, %)</th>
<th>Control group (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-28</td>
<td>6(27.3)</td>
<td>4(12.5)</td>
<td>7(20.6)</td>
</tr>
<tr>
<td>28-38</td>
<td>11(50.0)</td>
<td>16(50.0)</td>
<td>24(70.6)</td>
</tr>
<tr>
<td>38-48</td>
<td>3(13.6)</td>
<td>11(34.4)</td>
<td>3(8.8)</td>
</tr>
<tr>
<td>Above 48</td>
<td>2(9.1)</td>
<td>1(3.1)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>22(100)</td>
<td>32(100.0)</td>
<td>34(100.0)</td>
</tr>
</tbody>
</table>

Table 2 shows that the prevalence of anaemia in HIV positive individuals was significantly highest (66.7%) in individuals in the age group above 48 years. The difference in the degree of anaemia between this group and others was statistically significant.

Table 3. Comparison of mean concentrations of haematological parameters in the study groups.

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Anaemic HIV positive group</th>
<th>Non-anaemic HIV positive group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB</td>
<td>10.1±0.3</td>
<td>12.9±0.2</td>
<td>14.4±0.3</td>
</tr>
<tr>
<td>PCV</td>
<td>31.1±0.8</td>
<td>39.3±0.5</td>
<td>46.4±0.5</td>
</tr>
<tr>
<td>RBC</td>
<td>3.1±0.2</td>
<td>4.1±0.1</td>
<td>4.4±0.1</td>
</tr>
<tr>
<td>WBC</td>
<td>4.9±0.4</td>
<td>5.6±0.4</td>
<td>6.0±0.4</td>
</tr>
</tbody>
</table>

Table 3 displays that the mean concentrations of HB, PCV and RBC were significantly lower in the anaemic and non-anaemic HIV positive individuals when compared to the control group (HIV- negative persons). However, there was no significant reduction in the mean concentration of WBC in the HIV positive anaemic and non-anaemic groups when compared to the control group.

Table 4. Comparison of mean cytokine concentrations in HIV positive and control groups.

<table>
<thead>
<tr>
<th>Cytokine (pg/ml)</th>
<th>HIV positive group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>13.3±7.9</td>
<td>30.1±7.9</td>
</tr>
<tr>
<td>TNF-α</td>
<td>121.7±37.6</td>
<td>195±45.1</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>30.5±8.5</td>
<td>18.4±1.4</td>
</tr>
</tbody>
</table>

Table 4 shows that the mean levels of IL-1α and TNF-α were significantly lower in HIV positive group, while the higher level of IFN-γ obtained for HIV positive individuals was not significantly different from the control group.

Table 5. Pearson’s correlation between haematological parameters and pro-inflammatory cytokines HIV positive groups.

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Cytokines HIV positive group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB</td>
<td>IL-1α 0.14</td>
<td>IFN-γ 0.13</td>
</tr>
<tr>
<td></td>
<td>PCV 0.06</td>
<td>TNF-α 0.06</td>
</tr>
<tr>
<td></td>
<td>RBC -0.06</td>
<td>IL-1α 0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFN-γ 0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNF-α 0.01</td>
</tr>
</tbody>
</table>

Table 5 shows the correlation between haematological parameters and pro-inflammatory cytokines in HIV positive groups.
Table 5 shows weak correlations between haematological parameters and the three cytokines in HIV positive group when compared to the control group.

Table 6. Comparison of haematological parameters in study subjects by treatment type.

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>ZLN (n=12)</th>
<th>TLE (n=29)</th>
<th>TNP (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB</td>
<td>12.4±0.5</td>
<td>12.9±0.3</td>
<td>12.9±0.3</td>
</tr>
<tr>
<td>PCV</td>
<td>35.2±1.3</td>
<td>37.9±0.8</td>
<td>34.0±1.9</td>
</tr>
<tr>
<td>RBC</td>
<td>3.6±0.2</td>
<td>4.5±0.1</td>
<td>4.1±0.2</td>
</tr>
<tr>
<td>WBC</td>
<td>5.6±0.6</td>
<td>5.5±0.3</td>
<td>4.1±0.2</td>
</tr>
</tbody>
</table>

In table 6 displayed, there was a significant difference in the mean concentrations of RBC and WBC across the different treatment groups (ZLN, Nevirapine (ZLN) combination, Tenofovir-Lamuvudine-Efavirenz (TLE) combination and treatment naïve patients (TNP)) while no significant difference (p >0.05) was observed in mean concentrations of HB and PCV across the different groups.

Table 7. Comparison of cytokine concentrations in study subjects by treatment type.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>ZLN (n=12)</th>
<th>TLE (n=29)</th>
<th>TNP (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>13.8±3.6</td>
<td>13.8±2.5</td>
<td>11.7±1.1</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>25.4±10.2</td>
<td>39.1±15.1</td>
<td>18.2±3.4</td>
</tr>
<tr>
<td>TNF-α</td>
<td>126.5±74.2</td>
<td>139.1±74.2</td>
<td>112.0±55.1</td>
</tr>
</tbody>
</table>

There were positive correlations between IFN-γ and HB in TLE group but negative correlations in other two groups. Also, there were positive correlations between IFN-γ and WBC in ZLN group but negative correlations in other two groups.

5. Discussion

Anaemia is not uncommon in HIV infected individuals. Highly active antiretroviral therapy (HAART) affects HIV positive individuals either positively or negatively. The positive effects of HAART include: reduction of viral replication, potential healthy life style and reduction of mortality and morbidity rate thereby increasing survival rate. Conversely, the adverse effects include the impairment of haematopoiesis thereby causing a reduction in the development of blood cell lines and thereby leading to anaemia [22]. This study was carried out on HIV infected individuals This may be attributed to the extra blood loss during monthly menstruation and childbirth as also suggested by previous workers [24, 25].

Furthermore, the prevalence of anaemia was observed to be highest in the age group above 48 years. In contrast to these findings, other workers [26] observed highest incidence of anaemia in the age group ≥ 60 years and suggested that anaemia increased with age in HIV infected persons. The difference observed in this study may be due to number of subjects in the age group above 48 years.

In this study, lower mean concentrations of haemoglobin, packed cell volume, red cell count and white cell count were observed in HIV subjects than in the control group (HIV negative persons). These findings are in support of earlier reports and suggestions that suppression of the bone marrow and depletion of immune cells by HIV itself may have contributed to the low haematological parameters observed [6, 27, 28]. Also, the effect of HAART (Zidovudine combination) on the bone marrow may have contributed to these reductions [29].

In HIV infection, plasma cytokine levels are altered from the supposed normal level found in apparently healthy individuals that are not infected by HIV [21]. The mean levels of IL- 1α and TNF- α observed in this study were significantly reduced in HIV infected persons when compared to the control group. This decrease may be as a result of reduction in the immune cells themselves or impaired functions of cells secreting the cytokines [17]. It may also be that both cytokines are synergistically secreted by the same process [30] or that they augment each other’s secretion [31]. The reduction of TNF-α may also be as a result of its rapid binding to TNF-α receptor and its subsequent removal from circulation due to its short half-life.
Increased mean levels of IFN-γ level was observed though not statistically significant when compared to the control group. The increment supports a finding of sustained rise in IFN-gamma after the commencement HAART [33]. The depressed immune system caused by low CD4+ T cells numbers and rapid stimulation of the secretion of IFN-γ by HIV itself may contribute to cause the increased IFN-γ levels [34]. This result is in contrary to that observed by [18].

Weak correlations were observed between the haematological indices (haemoglobin, packed cell volume and red blood cell count) and pro-inflammatory cytokines (IL-1α and IFN-γ). This may be attributed to the depletion of IL-1α that play a major role in hematopoietic activities by HIV itself [17]. Also, increased IFN-γ might have caused viral proliferation that reduced the normal physiologic response to anaemia and regulatory cytokines that maintain normal haematopoiesis [8]. However, the mechanism for this action is yet unknown.

There was a statistically significant difference in the mean concentrations of red blood cells and white cells across the different treatment groups with Zidovudine- Lamuvidine-Nevirapine (ZLN) combination, Tenofovir-Lamuvidine-Efavirenz (TLE) combination and treatment naive patients (TNP) in HIV infected persons. The difference may have occurred due to the effects of Zidovudine- based regimen on the bone marrow which include the intoxication and suppression of the bone marrow [10, 29].

There were no significant differences observed between the mean concentrations of the three pro-inflammatory cytokines studied of patients on HAART and the means of treatment naive patients. We therefore postulated that the pro-inflammatory cytokines were not affected by HAART.

There were positive correlations between IFN-γ and HB in TLE group but negative correlations in other two groups. We suggest that IFN-γ may be associated with haematoepisis in HIV patients on TLE regimen. Also, there were positive correlations between IFN-γ and WBC in ZLN group but negative correlations in other two groups. We postulate that ZLN regimen enhances IFN-γ to stimulate immune response in HIV infected subjects.

There is still a dearth of knowledge in the role played by cytokines especially pro-inflammatory cytokines, in the pathology of HIV infection. Studies are therefore required in this area to ascertain their specific roles. Further studies are necessary to ascertain the effects of the different HAART regimens on pro-inflammatory cytokines in people living with HIV.

6. Conclusion

This study has shown that the dysregulated pro-inflammatory cytokine levels caused by HIV infection played a role in causing anaemia in HIV infection. Also, this study showed that IFN-γ may be associated with haematoepisis and enhanced immune response in HIV patients on TLE and ZLN regimen respectively.

References


