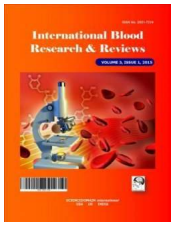

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Impaired Appropriateness of Erythropoietin in Anaemic HIV Infected Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AOT and ASA contributed to conceptualization, study design, data analysis, literature searches, recruiting of patients and controls and paper write-up. Authors OSO and EAA contributed to literature searches and review of manuscript. Authors AOA, AA and AAO performed study supervision and review of manuscript and author ASA contributed to study supervision and final review of manuscript. All authors read and approved the final version of the manuscript.

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ABSTRACT

Background and Objective: Erythropoietin response to anaemia has been reported to be suboptimal in HIV infected anaemic patients. Among Africans, particularly Nigeria little or no reports have been published describing incidence, prevalence and correlation between erythropoietin and anaemia in HIV, and its effect on the course and outcome of management of HIV infected patients.

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This study was carried out to determine the degree of erythropoietin response in anaemic HIV infected persons.
Methods: A total of 120 subjects were studied. These comprised of the study group made up of 40 HIV infected treatment naive patients who had anaemia with Haemoglobin level <10g/dL and 80 control subjects who were age and sex matched. The controls consisted of 20 HIV infected non-anaemic individuals, 40 non-HIV infected anaemic individuals, and 20 non-HIV infected non-anaemic individuals. Blood samples were collected for haemoglobin estimation, white cell count and platelet count by automated counter. The CD4+ cell count was done by semi-automated flow cytometer. The viral load was quantitated using PCR- based diagnostic tests and serum erythropoietin level was estimated using ELISA technique. All patients gave written informed consent with ethical approval by the hospital ethics and research committee.
Results: The study groups consisted of 40 males and 80 females. The mean age of male HIV anaemic subjects (45.15±5.63, N=13) was significantly higher than that of the female HIV anaemic subjects (34.16±7.21, N=25) p=0.005. The mean CD4+ cell count of HIV non-anaemic subjects (530.55 cells/µL ±423.35) was significantly higher than HIV anaemic subjects (188.18 cells/µL ±157.09) (p=0.0009). Using regression equation the expected serum erythropoietin values for a given haemoglobin level in HIV subjects was estimated. The appropriateness of erythropoietin level was then determined and a ratio of <0.8 was considered inappropriate. Seventy one percent of the HIV anaemic subjects had erythropoietin response ratio less than 0.8.
Conclusion: There was a blunted erythropoietin response for the degree of anaemia in HIV compared with HIV negative subjects.

Keywords: Anaemia; HIV; treatment naïve; erythropoietin response.

1. INTRODUCTION

Anaemia is a significant clinical problem in human immunodeficiency virus (HIV) infected patients and is the most frequently encountered haematological complication in HIV and acquired immunodeficiency syndrome (AIDS) [1]. The prevalence of anaemia varies, affecting up to 30% of patients with asymptomatic HIV infection and as many as 90% of those with advanced HIV disease [2,3]. Although highly active anti-retroviral therapy (HAART) appears to be associated with a somewhat lower risk of anaemia, anaemia remains common in the HAART era [3]. The causes of anaemia may differ with the stage of HIV disease and anaemia may function as a biomarker for poor outcomes in HIV [4,5,6].

Early in the HIV pandemic, it was recognized that anaemia was a prognostic marker of disease progression or death, independent of CD4+ cell count and viral load [7]. This observation remains true in the HAART era [3]. Additionally, anaemia impacts a range of dimensions of quality of life [8] most commonly due to its association with fatigue.

Among the several mechanisms that lead to HIV-associated anaemia, low endogenous erythropoietin (EPO) has been repeatedly reported. Thus it is speculated that a blunted EPO feedback mechanism contributes substantially to the pathogenesis of anaemia in HIV infected patients [4]. Other reports have also stated that the use of recombinant human EPO has been shown to be of immense benefit in the clinical status of patients [9,10]. A review by Ifudu of the State University of New York observed that HIV-associated anaemia can often be corrected with EPO therapy, which is safer than blood transfusion [10].

EPO is produced by the kidney in response to hypoxia. EPO regulates erythroid cell proliferation centrally. EPO expression is inversely related to tissue oxygenation and haemoglobin (Hb) levels, and there is a semilogarithmic relation between the EPO response (log) and the degree of anaemia (linear) [10]. EPO responses in anaemia of chronic disease are inadequate for the degree of anaemia in most, but not all, conditions [11,12]. Recombinant human EPO (rHuEPO) is the mainstay of treatment of hypoproliferative anaemia [7]. This led to the hypothesis that HIV associated anaemia may be at least partly due to relative hypoerythropoietinaemia.

This study determined the degree of EPO response in HIV infected subjects with anaemia.

2. MATERIALS AND METHODS

The study was carried out on a total of 120 subjects aged 18-66 years. They comprised of 40 HIV infected treatment naïve patients who had anaemia with Hb level <10g/dL (study group) and 80 control subjects who were age and sex matched. The controls were made up of 20 HIV infected non-anaemic individuals (control A), 40 non-HIV infected anaemic individuals (control B) and 20 non-HIV infected non-anaemic individuals (control C). The HIV infected patients were recruited from the HIV clinic during their clinic visit while the anaemic non-HIV infected individuals were recruited from the outpatient department of the hospital and the non-HIV infected subjects who had no anaemia were recruited from members of staff of the Lagos University Teaching Hospital (LUTH). The HIV status of all participants was confirmed from voluntary counseling and confidential testing. All patients who gave written informed consent were recruited and studied. The study was approved by the Ethics and Research committee of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos.

Exclusion criteria were pregnant females, patients on chemotherapy, patients with any medical condition that can cause anaemia such as chronic kidney disease, cancers, etc. and patients with peripheral blood film features of microcytic, hypochromic red cells/ anemia suggestive of iron deficiency anemia.

2.1 Procedure

Participants completed their questionnaires which had relevant information including age, sex, HIV risk factors, history of post- exposure prophylaxis, previous ARV experience and other medical conditions.

Venous blood (5 mls) was drawn from each participant using vacutainer containing anti-coagulant K3 EDTA for haematological indices which included Hb, packed cell volume (PCV), white blood cell (WBC) count & differentials, platelet count and red cell indices by automated counter, CD4+ cell count by semi-automated flow cytometer (Partec flow cytometer), peripheral blood film morphology was done by routine manual methods and HIV viral load was quantitated using PCR- based diagnostic tests by Roche amplicon. Venous blood (5 mls) was also collected into plain specimen tubes and allowed to stand at room temperature until clotted and the clot retracted. The blood samples in the plain specimen tubes were centrifuged and sera extracted. The sera (2 mls) were stored in cryogenic vials at -80ºC until time for analysis of serum erythropoietin levels when it was thawed at room temperature and mixed thoroughly before use. Serum erythropoietin level was determined by Enzyme Linked Immunosorbent Assay (ELISA) method using commercial assay kit manufactured by ALPCO diagnostics (Salem, New Hampshire). In this assay, calibrators, controls, or patient samples were simultaneously used as recommended by the manufacturers.

2.2 Statistical Analysis

Data was analysed using computer statistical software packages: SPSS for windows version 16.0, Microsoft excel 2007 and Epi Info version 3.5.1 2008 as applicable.

Data was expressed as means and standard deviation (standard error of sample) for continuous variables and frequency for categorical variables and associations between measured variables was tested using chi-square and students t-test for categorical and continuous variables respectively, and correlations were made. Analysis of variance was used where necessary.

EPO values, because they were not normally distributed, were log-transformed in the regression analysis.

The level of significance was set at $p < 0.05$.

3. RESULTS

A total of 120 subjects were recruited for this study. They consist of 4 categories of individuals which included 60 HIV infected patients (40 were anaemic and 20 were non-anaemic) and 60 HIV negative individuals (40 were anaemic and 20 were non-anaemic). The ages of the subjects were between 18 and 66 years. The study group consisted 40 males and 80 females.

The mean age of male HIV infected anaemic subjects (45.15±5.63) was significantly higher than that of female HIV infected anaemic subjects (34.16±7.21) p=0.005. Likewise the mean age for male HIV infected non-anaemic subjects (48.00±14.57) was higher than that for female HIV infected non-anaemic subjects (34.93±9.68) p=0.059. Table 1 shows the distribution of age and sex of the study subjects by category. In the HIV infected populations that

were anaemic, 57.1% of males were in age group 40-49 years and 87.5% of females were in age group 30-39 years while in HIV infected populations that were non-anaemic, 75.0% of males were in age group ≥50 years and 85.7% of the females were in age group 30-39 years. HIV infected males tended to be older than female HIV infected subjects. This is more manifest in anaemic HIV infected population where p=0.005. Unlike the non-anaemic group where p=0.267.

Table 2 shows that the mean CD4+ cell count of HIV infected non-anaemic subjects (530.55 cells/µL ±423.35) was significantly higher than that of HIV infected anaemic subjects (188.18 cells/µL ±157.09) (p=0.0009). Likewise the mean viral load of HIV infected anaemic subjects (455,244.89 copies/µL ±651,540.89) was significantly higher than that of HIV infected non-anaemic subjects (48,575.25 copies/µL ±113,531.29) (p=0.0005).

Table 1. Distribution of age and sex of subjects by category

Subjects category	Age group (years)	Sex of subjects (Frequency (%))		Total	Test	P value
HIV/Anaemic		**Male**	**Female**			
	<20	0(0)	1(100)	1(100)	X^2=14.99	0.005
	20-29	0(0)	4(100)	4(100)		
	30-39	2(12.5)	14(87.5)	16(100)		
	40-49	8(57.1)	6(42.9)	14(100)		
	≥50	3(100)	0(0)	3(100)		
n=38		13(34.2)	25(65.8)	38(100)		
Mean age		45.15±5.63	34.16±7.21		t=5.17	0.000
HIV/Non-anaemic						
	<20	0(0)	1(100)	1(100)	X^2=5.20	0.267
	20-29	1(25.0)	3(75.0)	4(100)		
	30-39	1(14.3)	6(85.7)	7(100)		
	40-49	1(25.0)	3(75.0)	4(100)		
	≥50	3(75.0)	1(25.0)	4(100)		
n=20		6(30.0)	14(70.0)	20(100)		
Mean age		48.00±14.57	34.93±9.68		t=2.01	0.059
Non-HIV/ anaemic						
	<20	4(75)	1(25)	5(100)	X^2=13.29	0.010
	20-29	4(26.7)	11(73.3)	15(100)		
	30-39	1(7.7)	12(92.3)	13(100)		
	40-49	0(0)	5(100)	5(100)		
	≥50	0(0)	2(100)	2(100)		
n=40		9(22.5)	31(77.5)	40(100)		
Mean age		23.88±5.15	32.90±9.34		t=3.75	0.000
Non-HIV/ Non-anaemic						
	20-29	4(80)	1(20)	5(100)	X^2=3.75	0.289
	30-39	7(58.3)	5(41.7)	12(100)		
	40-49	0(0)	1(100)	1(100)		
	≥50	0(0)	1(100)	1(100)		
n=19		11(57.9)	8(42.1)	19(100)		
Mean age		31.36±6.04	35.00±8.49		t=1.03	0.314

Table 2. Comparison of mean CD4+ cell count and viral load between HIV infected anaemic and non-anaemic treatment naive subjects

	HIV infected subjects		t-test	P value
	Anaemic	**Non-anaemic**		
Mean CD4+ cell count (SD) cells/µL	188.18(157.09)	530.55(423.35)	3.49	0.0009
Mean viral load (SD) copies/µL	455244.89(651540.89)	48575.25(113531.29)	3.69	0.0005

EPO was shown on frequency distribution curve to be skewed to the right. It is therefore not normally distributed (Fig. 1). The data become more normally distributed following natural logarithm transformation (Fig. 2).

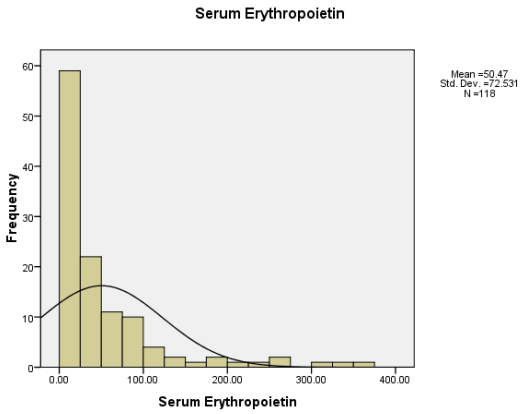

Fig. 1. Histogram of the frequency distribution of serum erythropoietin (EPO) levels for the study population

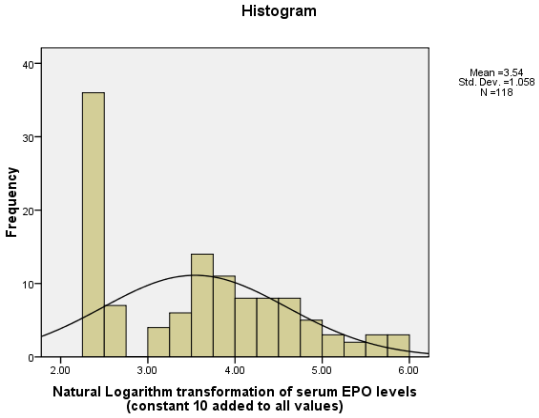

Fig. 2. Histogram of the frequency distribution of natural logarithm transformation of serum erythropoietin (EPO) levels for the study population

To determine appropriateness of EPO response in the anaemic HIV-infected subjects, the 40 HIV negative anaemic subject's serum EPO levels were plotted on a linear regression curve (Fig. 3) with their corresponding Hb values.

A strong negative correlation (Pearson's correlation coefficient= -0.4961 and p=.002) was established between serum EPO levels and Hb. A computer generated regression equation was y= -30.631x+309.96. Where y represents serum EPO levels in IU/mL and x represents Hb levels in g/dL. -30.631 represents the slope of the regressional curve and 309.96 is a constant. This regression equation was then used to estimate the expected serum EPO values for a given Hb level in HIV infected subjects. The appropriateness of EPO level was then determined by calculating a ratio of the observed (O) or measured EPO over the expected (E) or calculated EPO levels, O/E. A ratio of <0.8 was considered inappropriate [12]. Of the 38 HIV infected anaemic patients, 27 (71.05%) had EPO response ratio of <0.8.

The geometric mean for serum EPO level (mIU/mL) was 15.2±1.03, 23.83±1.12, 8.63±1.01 and 56.95±0.71 for HIV infected anaemic, HIV infected non-anaemic, HIV negative non-anaemic and HIV negative anaemic subjects respectively. These values were significantly different. F=10.43, p=0.000 (Table 3).

Correlation studies carried out for serum EPO showed significant negative relationship between serum EPO level and Hb (r=-0.268, p=0.003). There was no significant correlation between serum EPO levels and CD4+ cell count (r=0.075, p=0.572) and viral load (r=-0.083, p=0.537).

However there was a significant positive correlation between CD4+ cell count and Hb level (r=0.544, p=0.000) as well as a significant negative correlation between Hb level and viral load (r=-0.083, p=0.020).

There was also a significant negative correlation between CD4+ cell count and viral load (r=-0.299, p=0.025).

Table 3. Comparison of geometric mean and SD of the natural logarithm transformation (LN) of erythropoietin (EPO) in the subjects

	HIV subjects		Non-HIV subjects		Total	F	P value
	Anaemic	Non-anaemic	Anaemic	Non-anaemic			
Geometric mean EPO (SD)	15.2 (1.028)	23.83 (1.116)	56.95 (0.712)	8.63 (1.005)	118	10.43	0.000

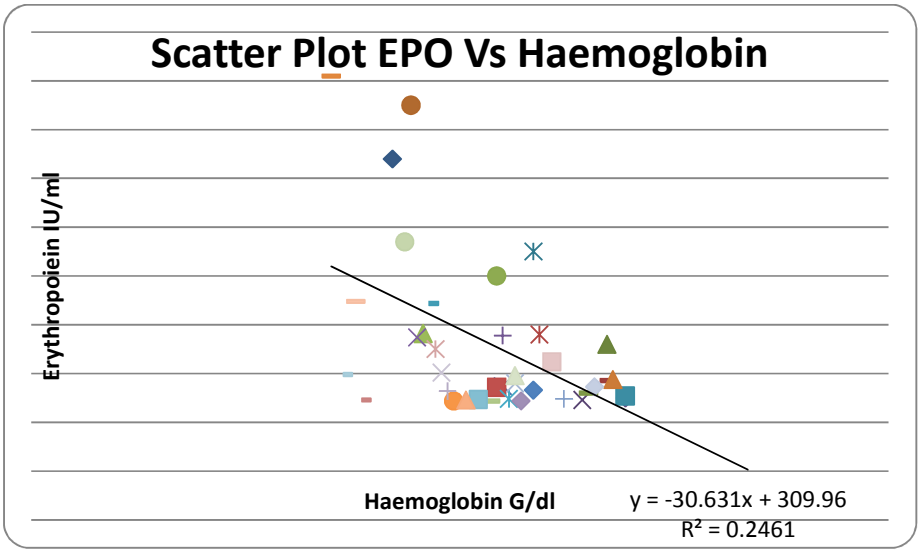

Fig. 3. Scatter plot of serum erythropoietin versus haemoglobin

4. DISCUSSION

Recovery from anaemia reduces the risk of disease progression to approximately the same levels as seen among patients who have never had anaemia [2].

This study confirms that the existence of an interaction between Hb levels and serum levels of EPO in treatment-naïve HIV infected patients with anaemia. It demonstrates that the expected rise in EPO levels in anaemic HIV-infected subjects does not occur compared with the rise in EPO levels in anaemic non-HIV infected subjects that have no other chronic disorders.

Similar to findings by Kelly et al. [13] male HIV infected anaemic subjects in this study were older than their female counterparts (p=0.005). This suggests that increase age difference between young women and their male sexual partners is a significant risk factor for HIV infection. These observations suggest that high HIV prevalence in younger women could be caused in part by transmission from older male partners. This trend as observed in our study may be connected to the fact that younger women in our society tend to look up to older men for relationship and marriage.

The non-HIV infected anaemic subjects had an expected EPO rise whereas the HIV infected anaemic subjects showed no significant expected rise in serum EPO levels. In this study, 71% of the HIV infected patients with anaemia had an EPO response ratio <0.8. This shows that there is an inappropriate EPO response to anaemia in HIV infected patients.

Physiologically, decreasing blood Hb concentrations are followed by increasing levels of EPO. The finding of lower than expected EPO response in HIV infected anaemic subjects is consistent with the findings that there is blunted response to EPO in HIV infected patients who have anaemia [4,14] and this non-response is documented in other literatures. For example, Camacho et al. [14] found a failure of appropriate EPO response in anaemic patients with advanced HIV infection. This study corroborates other findings which suggest that a blunting of the EPO response may be involved in the pathogenesis of the HIV-related anaemia [14-17].

Several mechanisms have been proposed for the blunted EPO response in anaemic HIV infected patients and this include hypergammaglobulinaemia and defective humoral immunity [15,16,17], role of cytokines [18], and acute *P. falciparum* malaria infection in the tropics [19].

Although hypergammaglobulinaemia and defective humoral immunity are hallmarks of HIV infection [16], the blunted EPO response in HIV infection related to hypergammaglobulinaemia have been thought to result from increased plasma viscosity [20] and modulation of humoral immunity via CD27+ memory B cells [15]. The study by Nagase et al. [15] identified a reduction

of CD4+ T cells in patients infected with HIV as well as hypergammaglobulinaemia and suggested that CD70 expressed spontaneously or by activation on T cells of HIV-infected patients stimulates memory B cells via CD27 and promotes their differentiation into plasma cells, resulting in the elevation of serum Ig levels and the elimination of circulating memory B cells in HIV-infected patients. It was noted in our study that the Hb level on its own is a determinant of CD4 cell count and viral load, the CD4 cell count is however a stronger determinant, nevertheless, the EPO level is usually determined by the Hb level, not CD4 cell count or viral load.

Leowattana et al. [19] found that acute *P. falciparum* malaria associated anaemia causes blunted EPO response in adult patients with anaemia. Although not the focus of this study, the influence of acute *P. falciparum* malaria infection cannot be ruled out as a cause of the inappropriate EPO response found in this work, the study being conducted in a malaria endemic area. Hence, it could be hypothesized that the blunted EPO response seen in anaemic HIV infected patients could be due to their being prone to malaria and other infections as a result of their low immunity. This raises the need for malaria and other infections prevention and control in patients with HIV infection.

Other authors have linked the inappropriate EPO response seen in HIV anaemic patients to the role of cytokines especially TNF/TNF-R system suggesting that TNF/TNF-R system may impair EPO production in HIV-associated anaemia [18].

The lack of consensus on the exact pathogenetic mechanism of inappropriate EPO response in HIV anaemic patients has raised the need for more research along this line.

5. CONCLUSION

Anaemia is a common manifestation in HIV infected patients not on antiretroviral therapy. In HIV treatment naive patients, a CD4+ cell count less than 200 cells/µL was associated with increased risk of anaemia. There was a positive correlation between Hb and CD4+ cell count. The mean serum EPO level were higher in HIV infected anaemic subjects than in the non-HIV infected non-anaemic subjects, even though there was a blunted response that was lower than the expected for the degree of anaemia seen in HIV infection when compared with HIV negative anaemic subjects who had good serum erythropoietin (EPO) response.

There is a need for a larger study on EPO response in HIV infected anaemic patients in order to assert or discard previous hypothesized mechanisms.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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