Variable Influencing Parameters of Different Levels of Immunosuppression in HAART-naive HIV Subjects in Nigeria

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Abstract

Background and Objectives: Varying degrees of immunosuppression may be observed in human immunodeficiency virus (HIV) infected subjects. This study sought to evaluate the factors which might influence different levels of immunosuppression in this group of subjects.

Methodology: Immunosuppression, defined as CD4<500 cells/ml, was evaluated in treatment-naïve HIV subjects. Body mass index (BMI), 24 h urine creatinine (24HUCr), 24 h urine protein (24HUP), creatinine clearance (ClCr), hemoglobin (Hb) and CD4 cells count were determined and the data compared among the subjects who have different levels of immunosuppression, defined here as mild for CD4 350-499 cells/ml, moderate for CD4 200-349 cells/ml and severe for CD4<200 cells/ml.

Results: CD4 cells count 200-349/ml was prevalent in 31.3%, CD4 350-499/ml in 25.4% and CD4 ≥ 500 cells/ml in 122(31.0%) of the HIV subjects. Immunosuppression was significantly associated with BMI (df=9, p=0.008), 24HUCr (df=6, p=0.019) and anemia (df=9, p<0.001). None of these three variables, in addition to 24HUP, was a predictor of moderate immunosuppression (CD4 cells count 200-349/ml). However, BMI, ClCr, 24HUP and Hb were predictors of mild immunosuppression (CD4 350-499 cells/ml) (p=0.006, p=0.008, p=0.026 and p=0.003 respectively).

Conclusion: Variable levels of immunosuppression were prevalent in this study. Anemia, abnormal weight and renal damage were common but variable in subjects who have different levels of immunosuppression in the early stage of HIV infection.

Keywords: Levels of immunosuppression; CD4 cells count; Anemia; Abnormal weight; Renal damage; HIV; Nigeria

Introduction

Immunosuppression is an ominous factor in HIV infection. Nigeria, a Sub-Saharan African country, not only has a high prevalence of HIV infection but is also enmeshed in the web of problems inherent in late presentation of cases to healthcare centers [1]. As a result, HIV subjects may have developed some complications associated with disease progression, even on presentation [2,3].

Disease activity and progression are mired by immunosuppression in HIV infection [4-7]. Studies have shown some factors which might influence immunosuppression in HIV subjects and non-HIV individuals. [8-11]. These factors include body mass index (BMI), demography, genetics, behavior and sex workers, among others [8-11]. A study has also demonstrated an association between low CD4 (<200 cells/ml) and BMI, serum low density lipoprotein cholesterol, anemia, 24 h urine protein, as well as creatinine clearance in treatment-naïve HIV subjects [8]. The advent of opportunistic infections and opportunistic malignancies are associated with immunosuppression in HIV infection [2,3,6-8,12]. There was a paucity of studies that assessed different levels of immunosuppression in HIV infected individuals. This study was carried out to determine the factors that might influence different levels of immunosuppression, adjudged by different levels of <CD4 500 cells/ml, in the early stage of HIV infection.

Materials and Methods

In this cross-sectional study conducted in Federal Medical Centre, Owerri, Nigeria, in 2011, HIV-positive subjects were consecutively recruited. The hospital, a tertiary health institution, serves the state which has a population of about 3,927,563 [13].

Age range of 16-65 years and treatment-naïve HIV positive status were the inclusion criteria, whereas the exclusion criteria were adrenal disease, renal or terminal illnesses, malignancies and pregnancy. Each participant in this study gave informed written consent. The study was approved by the Ethics Committee of the hospital.

With the aid of a questionnaire, demographic and anthropometric data were collected from the subjects. Gender, age, place of origin and domicile of the subjects were obtained. BMI was determined as weight/height^2 (kg/m^2).

Clear instructions were given to all the subjects on how to collect 24 h urine sample. For each participant, day-time blood samples and spot urine samples were collected at the end of the 24 h urine sample collection [8,14-16].

From the random spot urine samples collected, spot urine creatinine (SUCr) was performed. Also from the 24 h urine samples collected, 24 h urine creatinine (24HUCr) and 24 h urine protein (24HUP) were performed. Hemoglobin (Hb), CD4 cells count and serum creatinine were performed on the blood samples collected. Other tests done from the blood samples were HIV screening and confirmatory tests. Creatinine was determined by modified Jeff's method and protein by photometric method. Creatinine clearance (ClCr) was determined [8,14-16].

Statistical analyses

The data were analyzed using SPSS version 17.0 (SPSS Int. Chicago, II, USA). The distribution and characterization of BMI, Hb 24HUCr and ClCr among the subjects with different levels of CD4 cells count were analyzed using cross-tabulation. For continuous variables, mean values and standard deviations were calculated and the means compared using ANOVA or two sample t-test. Categorical variables were compared using the nonparametric tests - Chi-squares. Multivariate linear regression analyses were used to determine the strength of variables to predict CD4 200-349 cells/ml, CD4 350-499 cells/ml and CD4 ≥ 500 cells/ml. All the tests were two-tailed and $p ≤ 0.05$ was taken as statistically significant [8,14,17,18]. The potential risk factors of different levels of <CD4 500 cells/ml evaluated were BMI, 24HUCr, 24HUP, ClCr, and Hb.

Definition of terms

Mild immunosuppression: CD4 350-499 cells/ml

Moderate immunosuppression: CD4 200-349 cells/ml

Severe immunosuppression: CD4<200 cells/ml

WHO classification was used to define BMI levels as follows: [8,14,19]

Underweight=BMI<18.5 kg/m^2

Normal weight=BMI 18.5-24.9 kg/m^2

Overweight=BMI 25.0-29.9 kg/m^2

Obesity class I=BMI 30.0-34.9 kg/m^2

Obesity class II=BMI 35.0-39.9 kg/m^2

Obesity class III=BMI ≥ 40.0 kg/m^2

However, in this study, obesity was defined as class I, class II and class III obesity added together.

Anemia was defined according to the WHO criteria [8,14,20,21].

No anemia: Hb>13.0 g/dl in males and Hb>12.0 g/dl in females.

Mild anemia: Hb 11.0-13.0 g/dl in males and Hb 11.0-12.0 g/dl in females.

Moderate anemia: Hb 8.0-10.9 g/dl in males and Hb 8.0-10.9 g/dl in females.

Severe anemia: Hb<8.0 g/dl in males and Hb<8.0 g/dl in females.

However, in this study, anemia was defined as Hb<13.0.0 g/dl in males and Hb<12.0 g/dl in females. Overall, in this study, anemia was defined as Hb ≤ 12.0 g/dl.

Results

In the subjects studied, majority (97.0%) were Igbos; females constituted 72.0%. Their mean age was 39+11 years. CD4 200-349 cells/ml was observed in 123 (31.3%%), CD4 350-499 cells/ml in 100 (25.4%) and CD4 ≥ 500 cells/ml in 122 (31.0%). The mean values of the variables were 26.2 ± 5.4 mg/kgm^2, 11.2 ± 1.8 g/dl, 1507 ± 781 mg and 91.42 ± 22.98 mls/min for BMI, Hb, 24HUCr and ClCr respectively.

Among those who were underweight (BMI<18.5 kg/m^2) the prevalence of CD4<200 cells/ml of 4.2% was significantly low compared to CD4 ≥ 500 cells/ml of 25.0%, and CD4 200-349 cells/ml of 50.0%. Overall, CD4 cells/mml<500 cells/ml, observed in 75.0% of all those who were underweight was significantly high (df=9, p=0.008) (Figure 1 and Table 1).
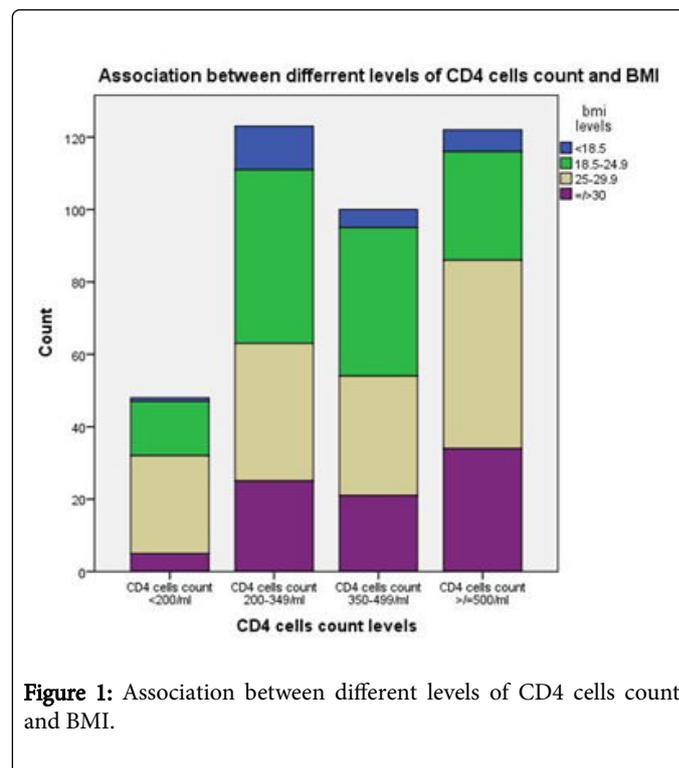

Figure 1: Association between different levels of CD4 cells count and BMI.

Variables	CD4 cells count levels (cells/ml) (no/%)				Λ2	Df	LHR	P value
	<200	200-349	350-499	≥ 500				
BMI (kg/m^2)<18.5	1(4.2%)	12(50.0%)	5(20.8%)	6(25.0%)	22.311	9	0.007	0.008
18.5-24.9	15(11.2%)	48(35.8%)	41(30.6%)	30(22.4%)				
25.0-29.9	27(18.0%)	38(25.3%)	33(22.0%)	52(34.7%)				

					Λ^2	df	p	LHR
≥ 30	5(5.9%)	25(29.4%)	21(24.7%)	34(40.0%)				
24HUCr <300 mg	0(0.0%)	0(0.05)	0(0.0%)	2(100%)	15.111	6	0.002	0.007
300-3000 mg	40(11.5%)	110(31.5%)	98(28.1%)	110(28.9%)				
>/=3000	4(16.7%)	8(33.3%)	0(0.0%)	12(50.0%)				
Hb (g/dl) ≥ 12.0	9(7.1%)	37(29.1%)	23(18.1%)	58(45.7%)	31.683	9	<0.001	<0.001
10.0-11.9	29(16.3%)	51(28.7%)	55(30.9%)	43(24.2%)				
7.0-9.9	8(9.8%)	35(42.7%)	20(24.4%)	19(23.2%)				
<7.0	2(33.3%)	0(0.0%)	2(33.3%)	2(33.3%)				
Λ^2=chi square, LHR=likelihood ratio, CD4=cluster of differentiation, BMI=body mass index, 24HUCr=24-hour urine creatinine, Hb=hemoglobin.								

Table 1: Distribution and characterization of variables at different levels of CD4 cells count in study subjects (n=393).

Among those whose weight were normal (BMI 18.5-24.9 kg/m^2), the prevalence of CD4 ≥ 500 cells/ml of 22.4% was significantly low compared to those who have CD4 350-499 cells/ml of 30.6% and CD4 200-349 cells/ml of 35.8% (df=xx, p=0.xx). However, CD4<200 cells/ml was observed in 11.2%, a very low prevalence among this group with normal weight. Similar results were observed in those who were overweight (BMI 25.0-29.9 kg/m^2) (Figure 1 and Table 1).

Twenty-four-hour urine creatinine<300 mg was observed only in those who have CD4 ≥ 500 cells/ml, demonstrating that dilute urine was significantly absent in those who have depressed immunity (df=6, p=0.019). Among those who have 24HUCr 300-3000 mg, the prevalence of CD4<200 cells/ml of 11.5% was significantly low compared to 31.5% and 28.1% in those with CD4 200-349 cells/ml and CD4 350-499 cells/ml respectively, df=6, p=0.019. Furthermore, the prevalence of CD4 ≥ 500 cells/ml of 50.0% observed in those whose 24HUCr was >3000 mg was high compared to 16.7% for CD4 <200 cells/ml, 33.3% for CD4 200-349 cells/ml and 0.0% for CD4 350-499 cells/ml. This however, demonstrated that immunosuppression declined as 24HUCr declined (Figure 2 and Table 1).

In the subjects whose Hb were normal (>12.0 g/dl), the prevalence of CD4<200 cells/ml of 7.1% was significantly low compared to 29.1% and 18.1% for those who have CD4 200-349 cells/ml and CD4 350-499 cells/ml respectively (df=9, p ≤ 0.001), indicating a low level of intense immunosuppression among those whose Hb were normal. In contrast, the prevalence of CD4 ≥ 500 cells/ml declined as Hb declined, 24.2% in Hb10.0-12.0 g/dl and 23.2% in Hb7.0-9.9 g/dl. Observed also was a significantly high prevalence (28.3% and 42.7% respectively) of CD4 cells 200-349/ml and CD4 cells 350-499/ml, among those who have Hb 10-12.0 g/dl and Hb 7.0-9.9 g/dl compared to those with CD4<200 cells/ml. A similar pattern was observed with CD4 350-499 and Hb10-12, Hb 7-9.9. This showed that progressive decline in immunity was significantly associated with worsening anemia, but those with the lowest immunity, however, did not have the highest prevalence of anemia (Figure 3 and Table 2).
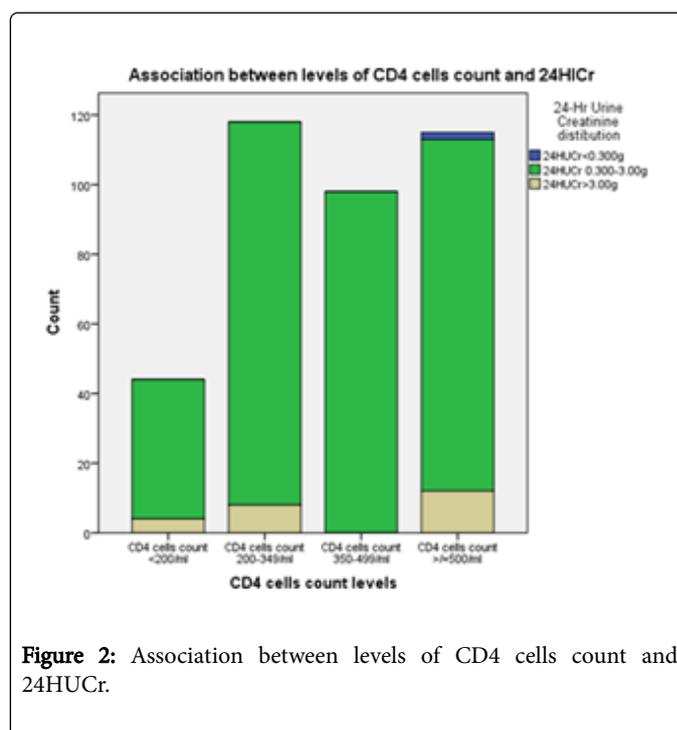

Figure 2: Association between levels of CD4 cells count and 24HUCr.

Significant but poor correlation was observed between CD4 cells count and BMI (r=0.122, p=0.015), Hb (r=-0.153, p=0.002), ClCr (r=-0.122, p=0.018) as well as 24HUP (r=-0.117, p=0.023). However, the correlation between CD4 cells count and 24HUCr was not significant, (r=-0.007, p=0.896).

Multivariate linear regression analyses showed that none of these variables studied predicted CD4 200-349 cells/ml, whereas CD4 350-499 cells/ml was predicted by BMI (p=0.006), ClCr (p=0.008), 24HUP (p=0.026) and Hb (p=0.003) (Tables 2-4). In contrast, Hb and 24HUP predicted CD4>500 cells/ml (p=0.013 and p=0.008 respectively).
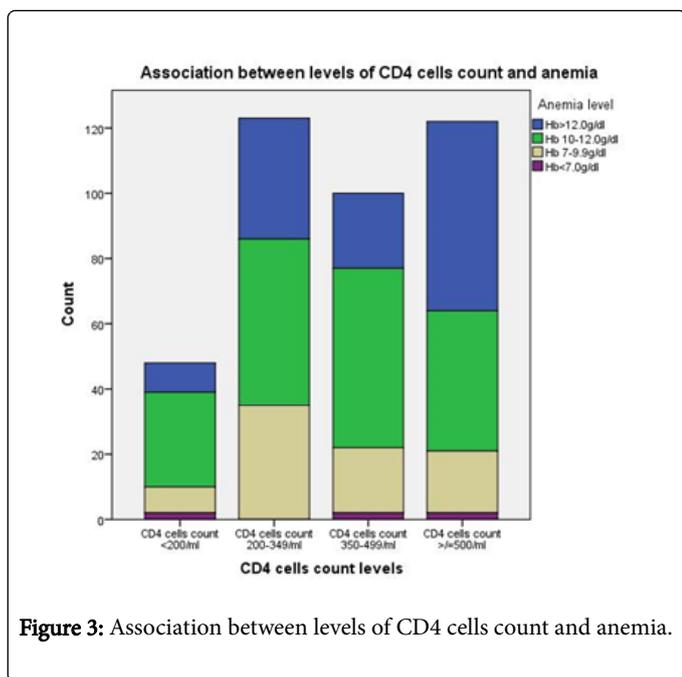
Figure 3: Association between levels of CD4 cells count and anemia.

Variables	Beta	T	P value	95% CI
Body mass index	-0.183	-1.24	0.926	-1.519-1.667
24 h urine creatinine	0.069	0.694	0.489	-6.906–14.358
Hemoglobin	0.189	1.8	0.075	-0.484–10.06
Creatinine clearance	-0.166	-1.806	0.073	-0.633–0.03
24HUP	0.116	1.224	0.223	-7.053–29.855
CI=Confidence Interval, R2=0.075, df=5, p=0.114				

Table 2: Multivariate linear regression of variables with CD4 cells count (200-349 cells/ml) in study subjects (n=123).

Variables	Beta	T	P value	95% CI
Body mass index	0.275	2.444	0.016	0.404–3.915
24 h urine creatinine	-0.089	-0.863	0.39	-9.844
Hemoglobin	-0.289	-2.553	0.012	-0.662
Creatinine clearance	-0.224	-2.155	0.034	-0.662
24HUP	0.216	2.27	0.026	7.548–113.260
CI=Confidence Interval, R2=0.203, df=5, p=0.001				

Table 3: Multivariate linear regression of variables with CD4 cells count 350-499 cells/ml in study subjects (n=100).

Discussion

Variable prevalence 31.3%, and 25.4% for CD4 200-349 cells/ml, CD4 350-499 cells/ml respectively observed in HIV subjects in this study were higher than the prevalence value of 11.2% for CD4<200 cells/ml reported by Anyabolu [8]. Together, both the Anyabolu study and this study show that in the spectrum of immunosuppression in HIV subjects, stepwise declining immunity was not unidirectional. Like any other Gaussian system, those who have moderate immunosuppression (CD4 200-349 cells/ml) peaked in prevalence, trailed by those who have mild immunosuppression (CD4 350-499 cells/ml) and those who have severe immunosuppression (CD4<200 cells/ml), as is demonstrated in this study.

Variables	Beta	T	P value	95% CI
Body mass index	-0.104	-1.113	0.268	-7.022–1.971
24 h urine creatinine	0.096	1.058	0.296	-13.916–45.790
Hemoglobin	0.226	2.523	0.013	3.705–30.860
Creatinine clearance	-0.131	-1.461	0.147	-2.033–0.307
24HUP	-0.251	-2.719	0.008	-348.958
CI=Confidence Interval, R2=0.143, df=5, p=0.005.				

Table 4: Multivariate linear regression of variables with CD4 cells count ≥ 500 cells/ml in study subjects (n=122).

In this study, majority (75.0%) of those who were underweight have CD4<500 cells/ml. In these underweight subjects who have immunosuppression, the prevalence of moderate immunosuppression (50.0%) was high compared to mild immunosuppression (2.8%) and severe immunosuppression (4.2%). Studies have shown an association between low CD4 cells count and underweight [8,22], but they did not evaluate the association CD4 might have with the different levels of immunosuppression, unlike this study. Declining weight is associated with infections and malnutrition in HIV subjects, especially in the developing nations [23]. However, this association is variable as shown in this study.

This study also shows that in obese HIV subjects, moderate immunosuppression (CD4 200-349 cells/ml) was high (29.4%), compared to mild (24.7%) and severe (5.9%). Obesity has been associated with immunosuppression, as shown in one study [8]. However, this study showed a Gaussian distribution of obesity with immunosuppression, peaking with moderate immunosuppression, and declining with mild and severe immunosuppression. It was also shown, in this study, that BMI predicted mild, but not moderate, immunosuppression, indicating a variable association between BMI and different levels of immunosuppression.

In this study, it was demonstrated that dilute urine [23] was significantly absent in the subjects who have reduced immunity, and further showed reduced urine concentration [23] in these immunosuppressed subjects, more pronounced among those who have moderate immunosuppression (33.3%), compared to those whose immunosuppression was mild (0.0%) and those severe (16.7%). This observation demonstrated that the ability to concentrate urine was not impaired by the degree of immunosuppression in these subjects. From literature search, there was a paucity of studies on the effects of levels

of immunosuppression on urine dilution and concentration. In one study, an association was demonstrated between urine creatinine and CD4 cells count, but like the second study by the same author, this association did not assess these effects at different levels of immunosuppression [8,23]. Nonetheless, some disease states, including those of the pituitary and kidneys may cause abnormalities of urine dilution and urine concentration [24].

This study showed that progressive decline in immunity was significantly associated with worsening anemia, but observed that those who have the lowest immunity, however, did not have the highest prevalence of anemia. Although a study has shown an association between low CD4 cells and anemia, it did not evaluate the different levels of immunosuppression, unlike this study [8]. Anemia is often associated with HIV disease progression, compounded by opportunistic infections and malnutrition [25]. Expectedly, anemia should increase as immunosuppression increases. This was not observed in this study.

In this study, ClCr, 24HUP and Hb were found to be predictors of mild immunosuppression (CD4 350-499 cells/ml). A study found an association between low CD4<200 cells/ml severe immunosuppression and ClCr as well as 24HUP [8]. However, the study did not assess CD4 different levels, differing from this study. Renal diseases are common in HIV infection and tend to increase with HIV disease progression, influenced by different levels of immunosuppression [15,26]. However, this study has demonstrated variable association between proteinuric renal damage and different levels of immunosuppression.

This study, overall, has shown that the association between different degrees of immunosuppression and abnormal weight, proteinuric renal damage as well as anemia was variable. Contrary to assumptions, these did not altogether increase with declining immunity.

Stakeholders involved in the management of HIV infection and its complications [27] should evaluate HIV subjects sequentially, on follow-up, for immunosuppression, determined by CD4 cells count, and search for anemia, abnormal weight and renal damage in those who have depressed immunity, irrespective of the degree of this immunosuppression [8].

Conclusion

Variable levels of immunosuppression were prevalent in this study. Anemia, renal damage and abnormal weight were common but variable at different levels of immunosuppression in HIV subjects in the early stage of the infection. There is a need for clinicians to assess immunosuppression in routine HIV clinical practice and to further search for abnormal weight, anemia and renal damage in those who have CD4 cells count <5000, with greater attention as CD4 cells count declines.

Limitation

An assessment of absolute HIV viral load was not done but would have added color to this study if it was evaluated. Further analyses of CD4 0-99 cells/ml and 100-199 cells/ml would have been of immense value, if they were included.

What is already known about this topic:

A: The prevalence of low CD4 cells count is high in treatment-naïve HIV subjects;

B: Abnormal weight, dyslipidemia and proteinuric renal damage were common among treatment-naïve HIV subjects who have low CD4 cells count;

C: Influencing parameters of different levels of immunosuppression have not been completely identified.

What this study adds to knowledge:

A: Variable degrees of immunosuppression are prevalent in treatment-naïve HIV subjects.

B: Anemia, renal damage and abnormal weight are common but variable at different levels of immunosuppression in HIV subjects in the early stage of the infection.

C: HIV subjects who have the highest immunosuppression did not have the highest degree of anemia or renal damage.

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