

Factors of Low CD4 Cells Count in Treatment-naïve HIV Subjects in Southeast Nigeria

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Abstract

Background and objectives: Human immunodeficiency virus (HIV) infection is a global healthcare problem. Low CD4 cells count, an index of immunosuppression, is associated with escalating activity and progression of HIV infection. The factors which influence low CD4 cells count have not been completely identified nor are they evaluated in routine clinical practice. The aim of this study was to determine the prevalence of low CD4 cells count and to evaluate the factors which might influence immunosuppression in treatment-naïve HIV subjects in Southeast Nigeria.

Methodology: This was a cross-sectional study involving treatment-naïve HIV subjects. Anthropometric and demographic data were obtained and CD4 cells count and other relevant investigations performed. The data were compared between those who have low CD4 cells count, defined, here, as CD4 <200 cells/ml, and those with CD4 ≥ 200 cells/ml. Potential risk factors of low CD4 cells count were determined.

Results: The mean age of the subjects was 39+11 years. Females were made up 283 (72.0%) and males 110 (28.0%). The median value of the CD4 cells count was 391. Low CD4 cells count was prevalent in 49 (12.5%) of the subjects. There was significant association between CD4 cells count and body mass index (df=2, p=0.017), as well as serum low density lipoprotein cholesterol (df=1, p=0.027) and anemia (df=3, p=0.025). Significant, but poor, correlation was observed between CD4 cells count and 24 h urine protein (r=-0.117, p=0.023), creatinine clearance (r=-0.122, p=0.018), as well as hemoglobin (r=0.224, p<0.001). Creatinine clearance was a predictor of low CD4 cells count, p=0.001.

Conclusion: The prevalence of low CD4 cells count was high in this study. Abnormal weight, dyslipidemia and proteinuric renal damage were common among treatment-naïve subjects who have low CD4 cells count.

Keywords: HIV; Low CD4 cells count; Abnormal weight; Dyslipidemia; Proteinuric renal damage

Introduction

Human immunodeficiency virus (HIV) infection is a global healthcare problem. The enormity of the burden of HIV infection is overwhelming, especially in Sub-Saharan African countries which account for about 70.0% of world HIV-infected individuals [1]. Mass media campaigns for HIV awareness from local authorities, World Health Organization and International Agencies have tremendously increased voluntary testing and counselling [2,3]. As a result, HIV subjects tend to report at the early stage of the infection [4].

However, despite these efforts and the relative availability of, and accessibility to, HIV centers, many HIV subjects still report late and, as a result, the diagnosis of HIV infection is often made in the course of these subjects presenting to hospital for other illnesses, mainly opportunistic infections that signal a state of immunosuppression [5,6].

Reduced immunosuppression is known to occur in many disease states, including HIV infection [7,8]. In HIV infection, disease activity and progression highly correlate with low CD4 cells count [9-12]. Low CD4 cells count has been used as a marker for the initiation of antiretroviral therapy (ART): CD4 <350 cells/ml for subjects in clinical stage iii and CD4 <200 cells/ml for any subject irrespective of clinical stage [13]. Low CD4 (CD4 <200 cells/ml), alongside HIV viral load, is therefore of immense clinical significance as it has been shown to be associated with batteries of opportunistic infections and opportunistic malignancies [7,10].

Some factors associated with low CD4 cells count have been identified. These include demographic and genetic factors, low body mass index (BMI), commercial sex workers, and behavioral factors, all

in HIV-negative individuals [8,14-17]. In HIV-infected individuals, it has been demonstrated that low CD4 cells count is a predictor of AIDS syndrome and disease progression. In addition, low CD4 cells count is associated with the advent of opportunistic infections and opportunistic malignancies [9-12].

There is a paucity of studies on CD4 cells count and the factors which influence low CD4 cells count in both the general population and HIV subjects in Nigeria. This has prompted us to embark on this study to evaluate CD4 cells count and to identify the factors which might influence low CD4 cells count in treatment-naïve HIV subjects with a view to instituting measures to stem down immunocompromise and poor clinical outcomes in the early stage of HIV infection.

Materials and Methods

This was a cross-sectional study of 393 patients recruited consecutively from the HIV Unit in Federal Medical Centre (FMC), Owerri, Southeast Nigeria. This study was conducted between April and August 2011. The hospital sub-serves the state and also receives

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referrals from the neighboring states. Owerri, where the hospital situates, has a local municipal population of about 125,337, whereas the state population is conservatively put at 3,927,563 [18].

The criteria for inclusion in this study were age range of 16-65 years and treatment-naïve HIV-positive status. Pregnancy, adrenal disease, renal or terminal illness and malignancy were the exclusion criteria. From each of the study subjects, informed written consent was obtained. The Ethics Committee of the hospital gave approval for the study.

From each of the subjects anthropometric and demographic data were obtained with the aid of a questionnaire administered by our laboratory technicians who explained to them the aim of the study. The place of domicile and origin, gender and age of the subjects were obtained. Weight and height were taken and BMI rendered as weight/height² (kg/m²). Blood pressure was measured [19].

Clear instructions were given to all the subjects on how to collect 24 h urine sample. For each subject, a day-time random spot urine sample and blood samples were collected at the end of the 24 h urine sample collection [19-21].

From the random spot urine samples collected, spot urine protein (SUP), spot urine creatinine (SUCr) and spot urine osmolality (SUOsm) were performed. Also from the 24 h urine samples collected, 24 h urine protein (24HUP), 24-hour urine creatinine (24HUCr) and 24 h urine osmolality (24HUOsm) 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, fasting blood sugar and fasting serum lipid profile (FSLP) [total cholesterol, triglyceride, HDL, LDL]. Osmolality was determined by freezing point depression method using Precision Osmette 5002 osmometer, creatinine by modified Jeff's method and protein by photometric method. Creatinine clearance (ClCr) was determined [19-21].

Statistical Analyses

The data were analyzed using SPSS version 17.0 (SPSS Int. Chicago, II, USA). The distribution and characterization of the clinical and laboratory features 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 low CD4 cells count (CD4 <200cells/ml). All tests were two-tailed. P ≤ 0.05 was taken as statistically significant [19-23].

The potential risk factors of low CD4 cells count evaluated were SUCr, SUOsm, BMI, serum triglyceride, serum HDL, SCr, SUP, 24HUCr, 24HUOsm, ClCr, 24-hour urine volume (24HUV) and Hb.

Definition of Terms

WHO classification was used to define BMI levels as follows [19,24]; Underweight =BMI<18.5 kg/m², Normal weight =BMI 18.5 - 24.9 kg/m², Overweight =BMI 25.0 - 29.9 kg/m², Obesity class I=BMI 30.0-34.9 kg/m², Obesity class II =BMI 35.0 - 39.9kg/m², Obesity class III = BMI ≥ 40.0kg/m². However, in this study, obesity was defined as class I, class II and class III obesity added together.

Normal urine osmolality: 24HUOsm 300-750 mOsm/kg H₂O [19,25,26]

Dilute urine: 24HUOsm <300 mOsm/kgH₂O. Concentrated urine: 24HUOsm >750 mOsm/kgH₂O.

Anemia was defined according to the WHO criteria: [19,27,28] 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.

Desirable serum LDL: serum LDL <2.6 mmol/l. Borderline serum LDL: serum LDL 2.6 - 4.1mmol/l. High serum LDL: serum LDL >4.1mmol/l. Desirable serum cholesterol: serum cholesterol <5.2mmol/l. Borderline serum cholesterol: serum cholesterol 5.2 - 6.2mmol/l. High serum cholesterol: serum cholesterol >6.2mmol/l. Desirable serum triglyceride: serum triglyceride <1.7mmol/l. Borderline serum triglyceride: serum triglyceride 1.7 - 2.2mmol/l. High serum triglyceride: serum triglyceride >2.2mmol/l. Low serum HDL: serum HDL <1.0mmol/l. Desirable serum HDL: serum HDL ≥ 1.0mmol [19,29].

Results

This study evaluated CD4 cells counts in 393 treatment-naïve HIV subjects. Females constituted 72.0% while males made up 28.0%. The mean age of the subjects was 39 + 11 years. Majority (97.0%) of them were Igbos. The median value of the CD4 cells count was 391. The mean values of other variables are shown in Table 1. Low CD4 cells count (CD4 <200 cells/ml) was observed in 49 (12.5%) of the subjects, whereas 343 (87.5%) have CD4 cells count ≥ 200 cells/ml.

No significant association was observed between CD4 cells count and ClCr (df=1, p=0.296) as well as 24HUP (df=3, p=0.863) (Table 2).

There was significant association between CD4 cells count and BMI (df=2, p=0.017). Out of 48 subjects who have CD4<200 cells/ml count, 1(2.0%) has BMI<18.5 kg/m², 15 (30.6%) have BMI 18.5-24.9 kg/m², 28 (57.1%) have BMI 24.9-29.9kg/m², whereas 5 (10.2%) have BMI ≥ 30.0 kg/m². This showed that the prevalence of low CD4 cells count <200 cells/ml declined with underweight as well as with obesity (Table 2 and Figure 1).

Variables (mean ± SD)	HIV Subjects
Body Mass Index (kg/m ²)	26.2 ± 5.4
CD4 count cells/ml (Median)	391
Hemoglobin (g/dl)	11.2 ± 1.8
SUOsm (mOsm/kgH ₂ O)	464 ± 271
Spot Urine Protein(mg/dl)	11.89 ± 19.13
Spot Urine Creatinine (mg/dl)	137.21 ± 98.47
24-Hour Urine Protein (g)	0.187 ± 0.290
Serum Cholesterol (mmol/l)	3.8 ± 1.2
Serum Triglyceride (mmol/l)	1.2 ± 0.4
Serum HDL (mmol/l)	1.2 ± 0.3
Serum LDL (mmol/l)	2.3 ± 1.0
24-Hour Urine Creatinine (mg)	1507 ± 781
24HUOsmkgH ₂ O	564 ± 501
Creatinine Clearance (mls/min)	91.42 ± 22.98

SD: Standard Deviation; SUOsm: Spot Urine Osmolality; 24UOsm: 24-Hour Urine Osmolality.

Table 1: Characteristics of variables in treatment-naïve HIV subjects.

VARIABLES	Low CD4 cells count (<200 cells/ml) (n/%) N=49	CD4 cells count ≥ 200 cells/ml (n/%) N=343	Chi Square	df	LHR	P value
BMI (kg/m²) <18.5	1 (2.0%)	23 (6.7%)	10.241	3	0/013	0.017
18.5-24.9	15 (30.6%)	119 (34.7%)				
25.0-29.9	23 (57.1%)	122 (35.6%)				
≥ 30	5 (10.2%)	79 (23.0%)				
Hb (g/dl) >12.0	9 (18.4%)	117 (34.1%)	9.337	3	0.029	0.025
10.0 - 12.0	30 (61.2%)	148 (43.1%)				
7.0 - 9.9	8 (16.3%)	74 (21.6%)				
<7.0	2 (4.1%)	4 (1.2%)				
CICr (mls/min) ≥ 60	43 (12.5%)	300 (87.5%)	1.095	1	0.446	0.295
30-59	2 (6.2%)	30 (93.8%)				
24HUP(mg)<300	38 (11.5%)	292 (88.5%)	0.743	3	0.853	0.963
300-3499	7 (13.0%)	47 (87.0%)				
24HUOmsOm/kgH₂O <300	29 (12.0%)	213 (88.0%)	4.053	2	0.036	0.132
300-750	17 (15.3%)	94 (84.7%)				
>750	0 (0.0%)	22 (100.0%)				
FSLP (mmol/l)						
Chol T Des (<5.2)						
BorderL(5.2-6.2)						
High (>6.2)						
LDL Des (<2.6)	35 (71.4%)	288 (84.2%)	4.874	1	0.045	0.027
BorderL (2.6- 4.1)	14 (20.6%)	54 (79.4%)				
HDL Low (<1)	19 (14.1%)	116 (85.9%)	0.466	1	0.596	0.495
High (≥ 1)	30 (11.7%)	227 (88.3%)				
TG Des (<1.7)	43 (12.2%)	310 (87.8%)	1.338	3	0.743	0.720
BorderL (1.7-2.2)	4 (13.3%)	26 (86.7%)				
High (>2.2)	2 (25.0%)	6 (75.0%)				

LHR: Likelihood Ratio; BMI: Body Mass Index; CICr: Creatinine Clearance; 24HUP: 24-Hour Urine Protein; 23HUOsm: 24-Hour Urine Osmolality; Hb: Hemoglobin; FSLP: Fasting Serum Lipid Profile; LDL: Low Density Lipoprotein Cholesterol; HDL: High Density Lipoprotein Cholesterol; TG: Triglyceride; des: Desirable; Border: Borderline

Table 2: Distribution and Characterization of Variables with different Levels of CD4 cells count (n=393).

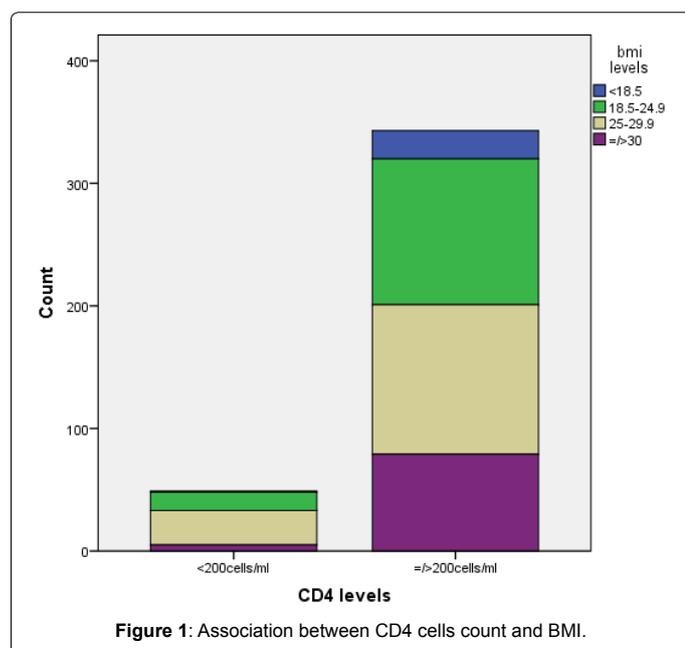


Figure 1: Association between CD4 cells count and BMI.

The prevalence of low CD4 cells count (<200 cells/ml) of 28.6% observed in those subjects who have borderline serum LDL (2.6-4.1 mmol/l) was significantly lower than 71.4% in those whose serum LDL was normal, df=1, p=0.027 (Table 2).

No significant association was observed between CD4 cells count and serum HDL (df=1, p=0.495) as well as serum triglyceride (df=3, p=0.720) (Table 2).

The prevalence of low CD4 cells count (<200 cells/ml) 81.6% observed in the subjects who have anemia was significantly higher than 18.4% in those who have no anemia, df=3, p=0.025 (Table 2 and Figure 2).

There was no significant association observed between CD4 cells count and 24HUOsm, df=2, p=0.134 (Table 2).

Significant but poor correlation was observed between CD4 cells count and 24HUP (r=-0.117, p=0.023), BMI (r=0.137, p=0.006), CICr (r= -0.122, p=0.018), as well as Hb (r=0.224, p<0.001) (Table 3). Conversely, there was no significant correlation between CD4 cells count and SCr, SUP, SUCr, SUOsm, 24HUV, 24HUCr, 24HUOsm, serum triglyceride, as well as serum HDL (Table 3).

Multivariate linear regression analysis showed that both CICr and Hb predicted CD4 cells count whereas only CICr predicted isolated low CD4 cells count (<200 cells/ml) in the study subjects (Table 4 and Table 5).

Discussion

The median CD4 cells count value of 391 found in this study is slightly higher than the 241 reported in a study in India [30] despite the background racial differences, but very much lower than the values found in normal healthy states [17]. This, together with the high prevalence of low CD4 cells count (CD4<200 cells/ml) of 12.5% tend to suggest that

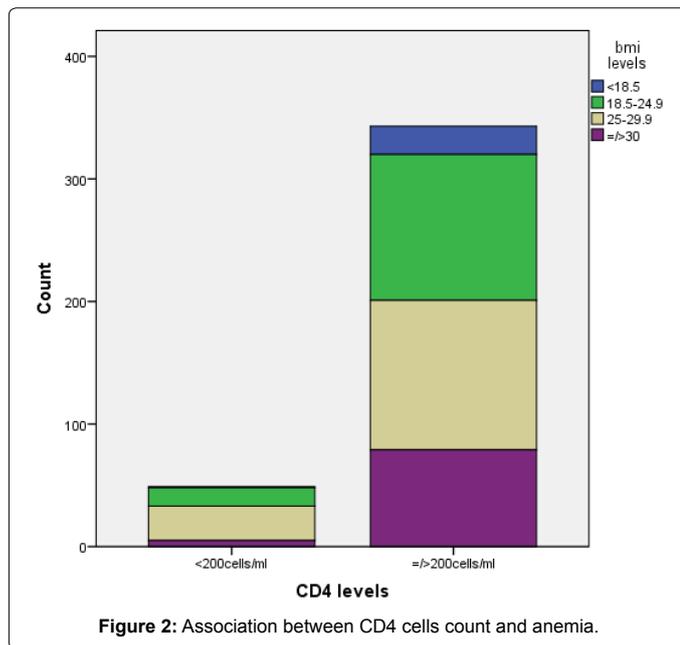


Figure 2: Association between CD4 cells count and anemia.

Variables	Correlation coefficient(r)	P value
Body mass index	0.137	0.006
Hemoglobin	0.224	<0.001
Spot urine protein	-0.069	0.171
Spot urine creatinine	0.015	0.763
Spot urine osmolality	0.037	0.467
24-hour urine protein	-0.117	0.023
24-hour urine creatinine	-0.019	0.707
24-hour urine volume	0.033	0.523
24-hour urine osmolality	0.073	0.160
Serum creatinine	<0.001	0.999
Creatinine clearance	-0.122	0.018
Serum triglyceride	0.035	0.488
Serum HDL	0.076	0.134

CD4: Cluster of Differentiation; HIV: Human Immunodeficiency Virus; HDL: High Density Lipoprotein Cholesterol

Table 3: Correlation of CD4 cells count with selected variables in treatment-naïve HIV-positive subjects (n=393).

Variables	Beta	T	P value	95% CI
Body mass index	0.051	0.959	0.338	-2.35 - -0.303
Hemoglobin	0.225	4.453	<0.001	13.925-35.949
24-hour urine protein	-0.076	-1.511	0.132	-123.747-16.218
Creatinine clearance	-0.131	-2.620	0.009	-2.046 - -0.292

CI: Confidence Interval, R²=0.076, df=3, p=0.002.

Table 4: Multivariate linear regression of variables with CD4 cells count in treatment-naïve HIV-positive Subjects (n=393).

Variables	Beta	T	P value	95% CI
Body mass index	-0.183	-1.240	0.222	-0.039 - 0.007
Hemoglobin	0.058	0.400	0.692	-0.025 - 0.037
24-hour urine protein	-0.006	-0.044	0.965	1.230 - 0.257
Creatinine clearance	-0.547	-3.667	0.001	-0.008 - -0.002

CI: Confidence Interval, R²=0.517, df=4, p<0.001

Table 5: Multivariate linear regression of variables with low CD4 cells count (<200 cells/ml) in treatment-naïve HIV-positive Subjects (n=49).

our study subjects have immunosuppression at onset. However, our study did not evaluate other indices of immunosuppression.

This study showed that low CD4 cells count significantly declined in prevalence with underweight as well as with obesity. This is in disagreement with the observation made in one study which noted that the prevalence of low CD4 cells count increased with low BMI [31]. Underweight is usually associated with infections and malnutrition in the developing countries [32], very overtly in the setting of HIV infection, both of which, expectedly, would cause a decline in CD4 cells count [22]. However, this was not observed in this study, suggesting that these subjects probably did not have much malnutrition or other co-infections.

In this study the prevalence of low CD4 cells count (<200 cells/ml) of 28.6% observed in those subjects who have borderline serum LDL (2.6-4.1 mmol/l) was significantly lower than 71.4% in those whose serum LDL was normal. This showed that dyslipidemia was associated with CD4 cells count abnormality. Dyslipidemia is commonly observed in subjects with higher socioeconomic status as well as in urban dwellers, and less in rural dwellers, majority of whom might have a sense of wellbeing, but paradoxically reduced immunity, as it has been shown that obesity impairs immunity [33].

This study did not observe any significant association between CD4 cells count and serum HDL as well as serum triglyceride. Nonetheless, dyslipidemia, which includes isolated low serum HDL, isolated serum triglyceride, or in combination with other abnormal serum lipids, is usually observed in conditions of over-nutrition, liver diseases and other chronic illnesses with depressed immunity [33].

The prevalence of low CD4 cells count (<200 cells/ml) 81.6% observed in the subjects who have anemia was significantly higher than 18.4% in those who have no anemia, as shown in this study. This observation is similar to that documented in another study [28]. Anemia is a catabolic state that might be associated with stressors that deplete CD4 cells [34,35]. In addition, anemia is associated with a lot of infection, especially in the developing countries, which might diminish the CD4 cells [36]. These, perhaps, might account for the high prevalence of low CD4 cells observed in our study, indicating also that these subjects might have a substantial level of anemia.

In this study, there was no significant association between CD4 cell count and 24HUOsm. There was a dearth of studies, from literature search, that evaluated the relationship between CD4 cells count and 24HUOsm. Stress may cause alterations in water intake, and the state of hydration. In addition, the kidney may be involved in the cause of HIV disease progression. Renal interstitial involvement may result in a salt-losing nephropathy. These may alter urine osmolality in a setting of low CD4 cells count [37]. These, however, were not observed in our study subjects.

Renal filtration function, determined by CICr has been shown to decline with the progression of HIV infection [20]. A study has also documented a high prevalence of chronic kidney disease in treatment-naïve HIV subjects. This study by Anyabolu et al. demonstrated that renal damage in this group of subjects involved both proteinuric renal disease defined by significant urine protein excretion, and renal damage defined only by impaired renal filtration function (CICr) [22]. The prevalence of low CD4 cells count noted in this study probably suggests that these subjects might have a measure of renal disease that might explain the association between the low CD4 cells count and CICr as well as 24HUP.

Serum creatinine is fairly regulated at a constant level and only rises with impaired renal function [38]. On the contrary, SUCr varies over a wide range of values in response to many factors that include protein intake, some medications, muscle mass, among others [25]. However, SUCr and 24HUCr may become impaired following renal disease in which urine creatinine excretion decreases [39]. This study, however, did not find a significant correlation between CD4 cells count and SCr, 24HUOCr as well as SUCr.

It was observed in this study that CD4 cells count has no significant correlation with 24HUV. There was a paucity of studies, from literature search, on the relationship between CD4 cells count and 24HUV. Some environmental conditions, some disease state and some physiologic state might influence urine volume [25]. These influencing factors of urine volume were not, however, evaluated in this study.

The myriads of the clinical implications of high prevalence of low CD4 cells count in treatment-naïve HIV subjects are dumbfounding and need to be addressed. Perhaps, the Leonardo Project as assessed and demonstrated by Ciccone et al. has shown that such an integrative and collaborative system, a multi-disciplinary, multi-level framework involving all the stake holders in the management of a chronic disease would also help in no small measure if applied to HIV subjects. Central to good outcomes in this system is a resource care manager, an anchoring coordinator, whose inputs would include empowerment of HIV patients through health education and implementation of all the recommendations of the different strata of clinicians involved in the management of HIV infection and its complications [40]. Those among HIV subjects who have dwindling, or overtly, low CD4 cells count, as in our study subjects, would be timely identified for evaluation that should include, among others, assessment for proteinuric renal damage, dyslipidemia, anemia, and abnormal weight, in the early stage of the infection. This care manager should coordinate the harmonious flow of patient care between primary care centers, HIV clinics, general practitioners and specialist clinicians for target patients' management outcomes.

Conclusion

The prevalence of low CD4 cells count was high in this study. Abnormal weight, dyslipidemia, anemia and proteinuric renal damage were common among treatment-naïve subjects who have low CD4 cells count. There is a need for clinicians to routinely evaluate CD4 cells count and to further search for abnormal weight, dyslipidemia, proteinuric renal damage and anemia in subjects who have low CD4 cells count <200 cells/ml at the early stage of HIV infection.

Limitations

This study did not assess the different levels of CD4 cells. This would have helped in also determining other factors that influence CD4 cells at different levels of immunosuppression.

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