Original Research Article

Immunological and Hematological profile of HIV patients on Anti-retroviral therapy in Port Harcourt, Rivers State, Nigeria.

ABSTRACT:

Aims: To profile CD4 count, TNFα levels and cytopenia in HIV seropositive patients on AR and also evaluate the prevalence of hematological cytopenia.

Study design: the present study is a cross sectional study and was carried out in the department of immunology, hematology and blood transfusion, department of pharmacology university of Port Harcourt and university of port-Harcourt teaching hospital, between May 2016 and July 2016.

One hundred patients (45 males and 55 females) were recruited for this study, patients included in this study were HIV positive and on anti-retroviral treatment for at least three months, they were not on any mind altering medications and were mentally sound and above 18 years 4 patients dropped out of the study.

The results: Hematological examination, CD4 count and serum TNF α levels were done. The prevalence of anemia was high (43.7%), neutropenia and leucopenia showed prevalence of 26.00% and 21.9% respectively. Prevalence of lymphopenia was 2.1%, whereas thrombocytopenia was 11.5%. A profile of immunological markers showed that mean CD4 was 395.81±273.046, high serum TNF α 65.33.20±58.52pg/ml was observed. WBC, lymphocyte and neutrophil were 4.82±1.38, 45.09±10.17 and 42.83±11.05 respectively, serum TNF α was increased while relative lymphocyte and neutrophil counts were decreased in patients with CD4 less than 200. Platelet count of participants in this study was 243.62±66.13 while RBC, PCV and hemoglobin was 3.82±0.75, 36.39± 4.10 and 12.00±1.806g/l respectively.

Conclusion: Patients with CD4 less than 200 showed increased TNF α, reduced CD4, total lymphocyte and neutrophil counts. Finding from this study has led to the recommendation that these parameters be used as biomarkers in monitoring patients and disease control in Nigeria.

1.0 INTRODUCTION

It has been shown that inflammation causes increased viral replication, increases the rate of virus entry and causes immune dysfunction in human immunodeficiency virus (HIV) infection, this indicates that immune activation is an important factor in the immune impairment associated with HIV/AIDS progression and the progression of the disease itself [1]. Studies in Europe and North America have shown that the constant replication of HIV virus is the main cause of the chronic inflammation that characterizes HIV patients1. The HIV epidemic observed in these places differs from the epidemic recorded in sub-Sahara Africa. Studies of Africans have shown persistent abnormal immune activation [2].

The devastating impact of the virus can be felt on the socioeconomic growth of developing countries since their productive young adults are infected. Developed countries should therefore encourage research on HIV in developing nations to reduce the impact of HIV on the economic growth.

CD4 count and HIV RNA concentration are very significant biomarkers of disease progression in HIV patients [3]. Nonetheless, some other factors can predict and influence the outcome of the disease [4]. Hematological abnormalities such as anemia, neutropenia and thrombocytopenia are commonly observed in HIV patients [5], also increased tumor necrosis factor alpha (TNF- α) can be observed. It therefore means that these markers can have been proposed as alternative markers in developing countries.

Various mechanisms that explain the depletion of CD4 cells have been proposed, this shoe because of the crucial role of CD 4 cells in immunity, therefore it is not surprising that its depletion has a devastating impact on host immunity.

Factors that can contribute in monitoring the progression of HIV infection may be important in explaining AIDS in Africa.

CD4 count has become such a useful marker for monitoring immune function in HIV infected patients, hence its extreme value in the management of HIV infection.] CD4 count is also a criterion in CDC/WHO classification of HIV infection this criterion is widely used to categorize patents for clinical management of the disease [6].

Tumor necrosis factor-alpha (TNF-α) is derived from blood cells and remarkable increased levels in the blood have been observed in HIV patients. A range of hematological manifestations is seen during the course of HIV in patents, the manifestation usually presents a great challenge in the management of HIV [7]. The most significant is anemia and then neutropenia and thrombocytopenia. They are generally caused by inadequate production and abnormal expression of cytokines [8].

The prevalence of leucopenia varies widely in patients with HIV, reported cases are between 10-15% [9].

The present study is aimed at evaluating prevalence of anemia, leucopenia, thrombocytopenia the levels of serum tumor necrosis factor alpha (TNF α) and CD4 count of patients on anti-retroviral therapy (ART).

The findings from this study will inform policy and practice as regards safe provision of anti-retroviral therapy (ART) to patients.

It will also present preliminary data regarding hematological manifestation and serum TNF-α level in this part of the Nigeria.

The relationship between various hematological manifestations CD4 TNF α will be investigated.

2. MATERIALS AND METHODS

2.1 Study area

This prospective observational study was carried out in the Department of Immunology, Faculty of Basic Medical Sciences in collaboration with the University of Port Harcourt teaching Hospital Rivers State. University of Port Harcourt teaching hospital is owned and operated by the federal, government of Nigeria. Permission and Ethical Clearance were obtained from the University of Port Harcourt teaching hospital ethics committee. Informed consent was obtained from patients by either signature or thumb print.

2.2 Study Population and Procedure

Minimum sample size was determined using the formula: $n = \frac{(z_{\alpha/2})^2 p(1-p)}{E^2}$

> Z=1.96 (95% Confidence interval
> p = 0.5
> E = 0.103
> z* p * (1−p) / E^2

Minimum sample size=90 (primary maker was the prevalence of immune failure defined as CD4 <200)

A total of 100 HIV patients were recruited into the study to accommodate for the fallout (4 Patients) and also meet the required minimum sample size for the study. A well evaluated and structured questionnaire was used to obtain information on demographic characteristics. The study population consisted of HIV patient on Anti-retroviral therapy (ART). Patients who started treatment within the last three months were not recruited. Patients were recruited based on the center for disease control (CDC 1993) revised classification for HIV infection G.3

INCLUSION CRITERIA

1) HIV positive.
2) On Anti-retroviral therapy (ART) for at least 3 months.
3) Not Terminally ill.
4) Ability to give informed consent
5) Not on any herbal, traditional or complementary medicine in the last 2 weeks prior to commencement of study

EXCLUSION CRITERIA

1) Less than 18 years old
2) Unable to give informed consent
3) Severely ill patients
4) Pregnant or planning to be pregnant in the next 4 months.
5) On traditional, herbal or complementary medicines.
6) On mind altering medications

Participants were properly informed about the study after which a consent form was signed by participants for documentation.

2.3 Blood Sample Analysis

About 4ml of venous blood was collected into a lithium heparin bottle by an experienced laboratory technologist from each subject for immunological and hematological investigation. Hematological parameters were determined using hematology analyzer (Mindray, China.), CD4 count was carried out using Partech cyflow machine (Cytech development Inc, Partech, Germany). Hematology laboratory analysis was carried out in Hematology Blood transfusion and Immunology department of the University of Port Harcourt Teaching Hospital (UPTH), Rivers state Nigeria. ELISA test was performed in the Chemical pathology department of the University of Port Harcourt Teaching Hospital (UPTH), Rivers state Nigeria

2.4 Statistical Analysis

Software program (SPSS version 20, SPSS Chicago) was used to analyze data. Mean and standard deviation was used to describe the data. Charts were also used to present data graphically.

2.5 Consideration

Ethical clearance for this research was obtained from the Ethical Committee of the University of Port Harcourt.

3. RESULTS AND DISCUSSION.

Table 1: Demographics of patients recruited for the study

Variables	Frequency (no. of occurrence)	Percentage ($\frac{frequency}{sample\ size} x\ 100$)
F	55	71.0
M	45	29.0
Educational status		
No formal education	11	2.8
Post graduate	11	17.6
Primary	23	13.4
Secondary	33	55.9
Tertiary	5	.8
Undergraduate	13	9.6
Occupation of participants		
Business man	11	13.1
Civil servant	13	17.1
Engineer	7	1.8
Farmer	6	4.0
Mechanic	1	.3

Seamstress	1	.3
Self Employed	23	14.1
Student	21	7.8
Tailor	1	.3
Teacher	1	.3
Trader	11	41.1
Income in Naira		
>50,000	28	17.1
10-30,000	18	31.2
10-30,001	14	2.5
30-50,000	17	34.0
5-10,000	19	15.1

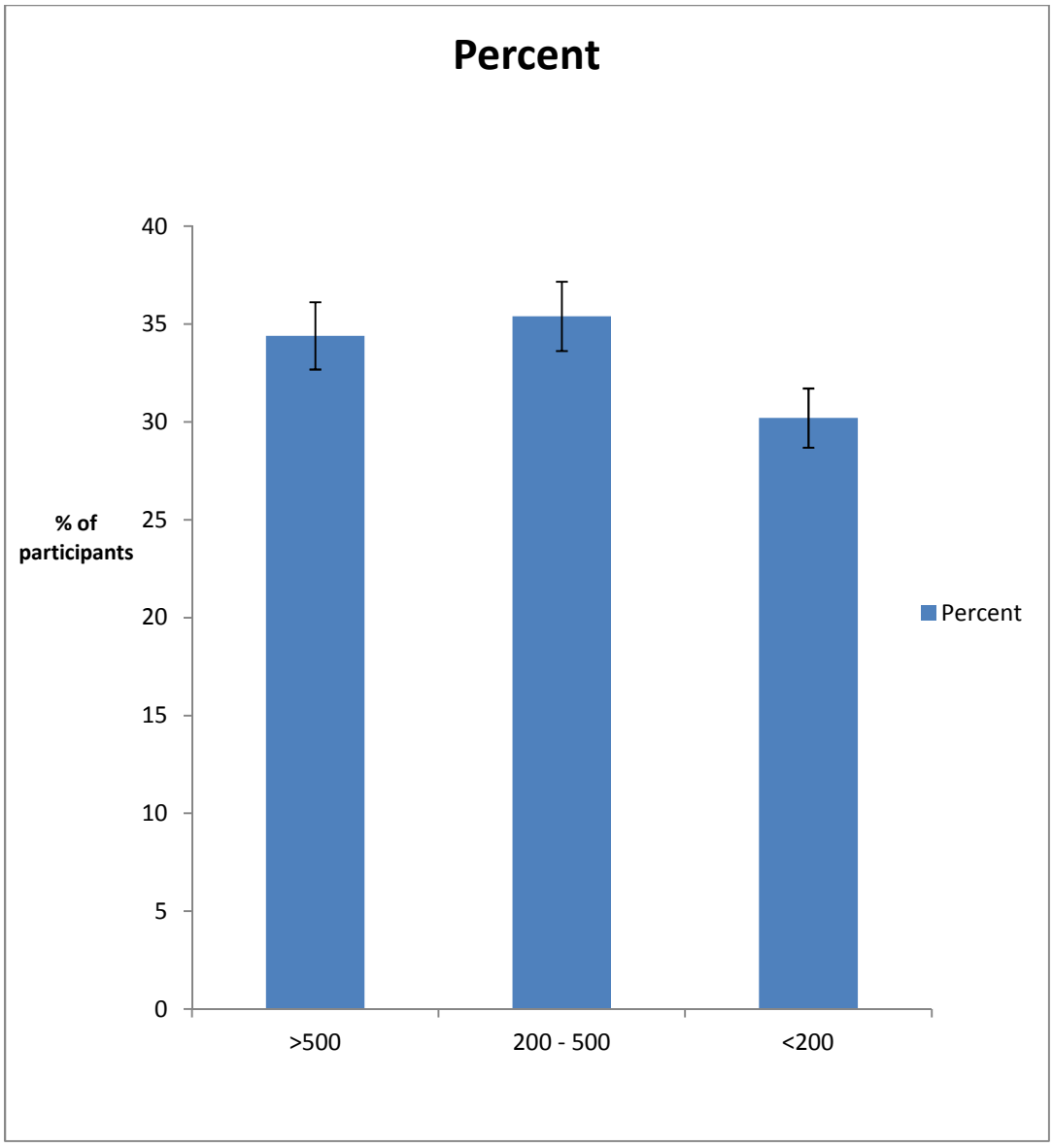

Figure 1 percentage of patients in various CD4 categories

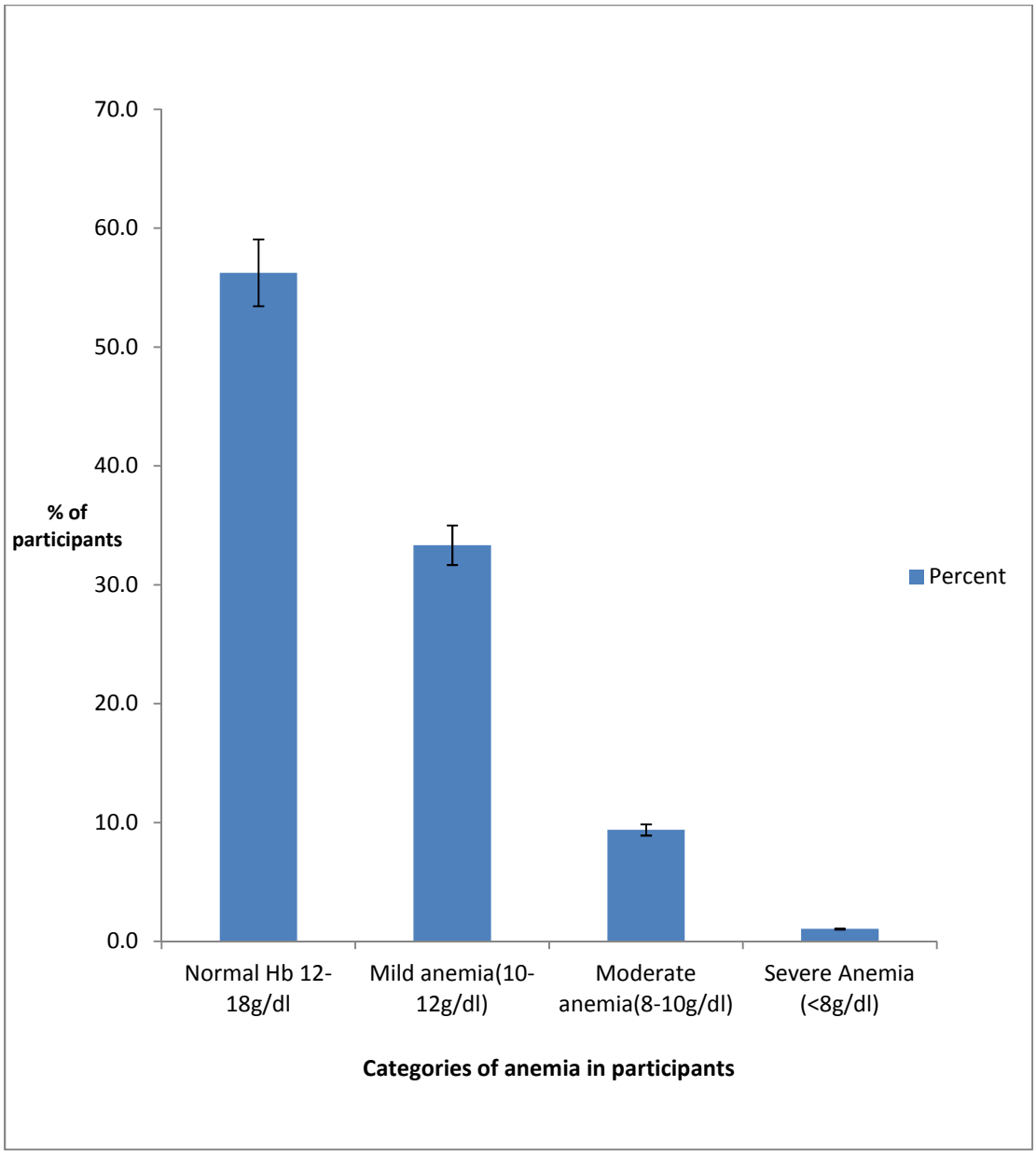

Figure 2 severity of anemia in participants

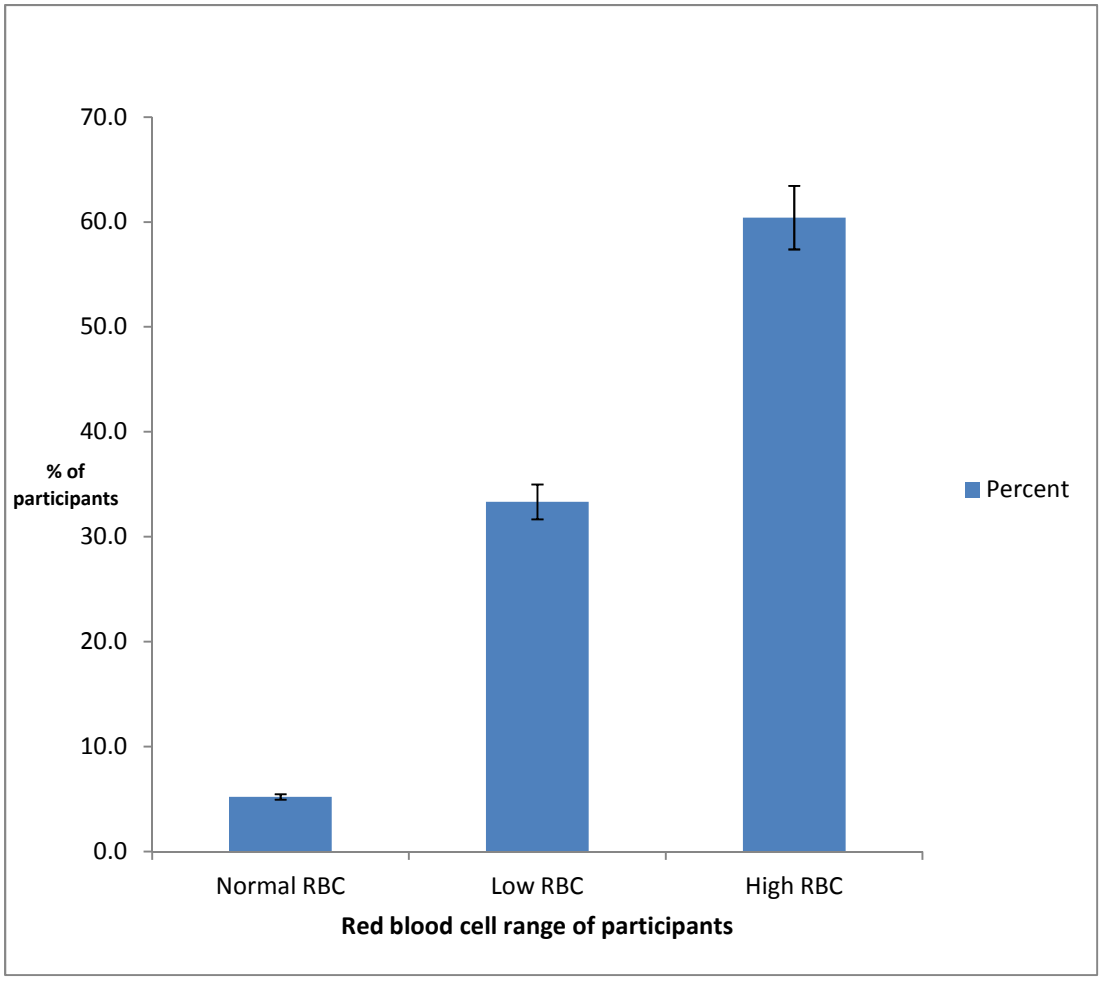

Figure 3 Red blood cell distribution of participants

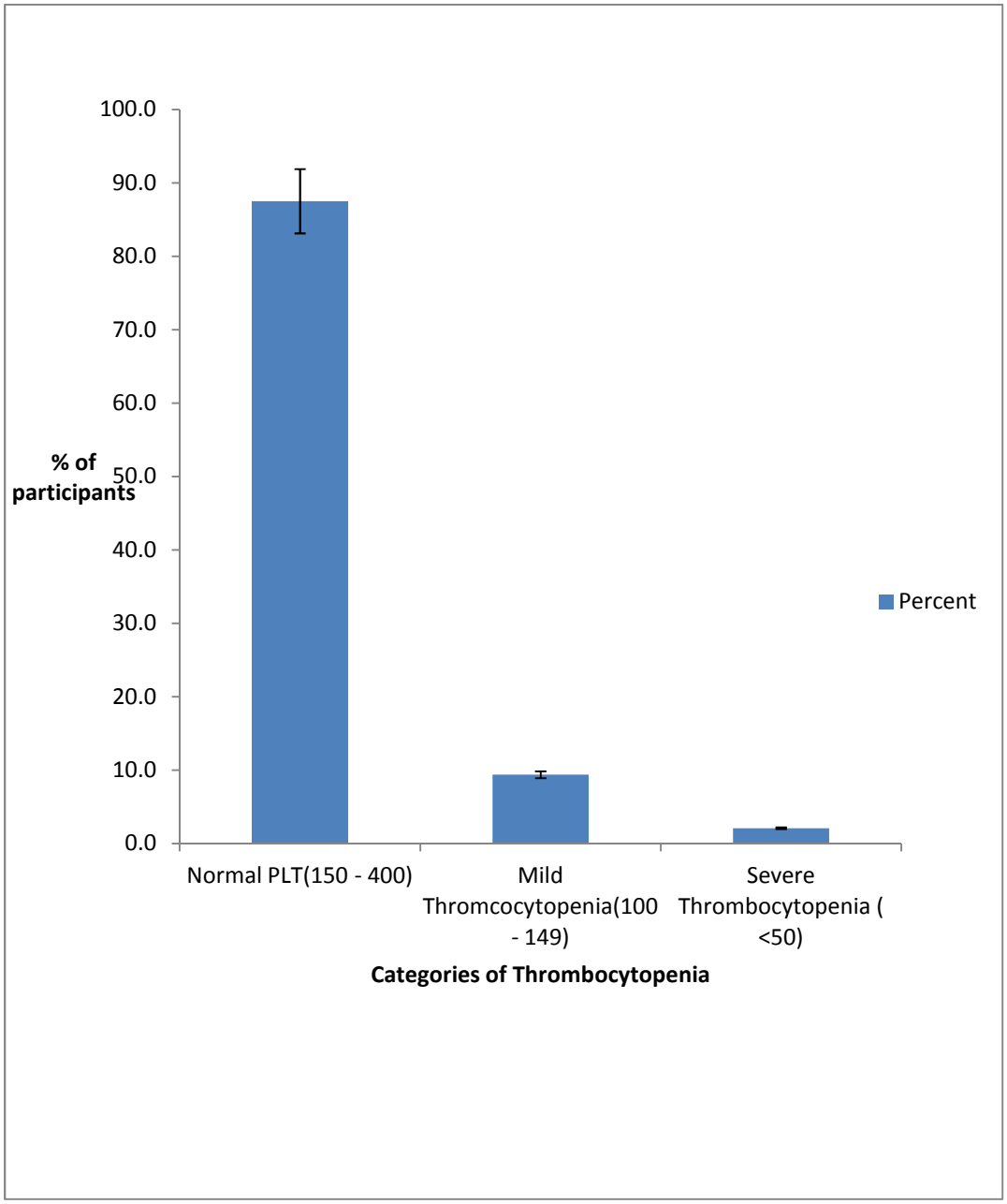

Figure 4 Prevalence of thrombocytopenia

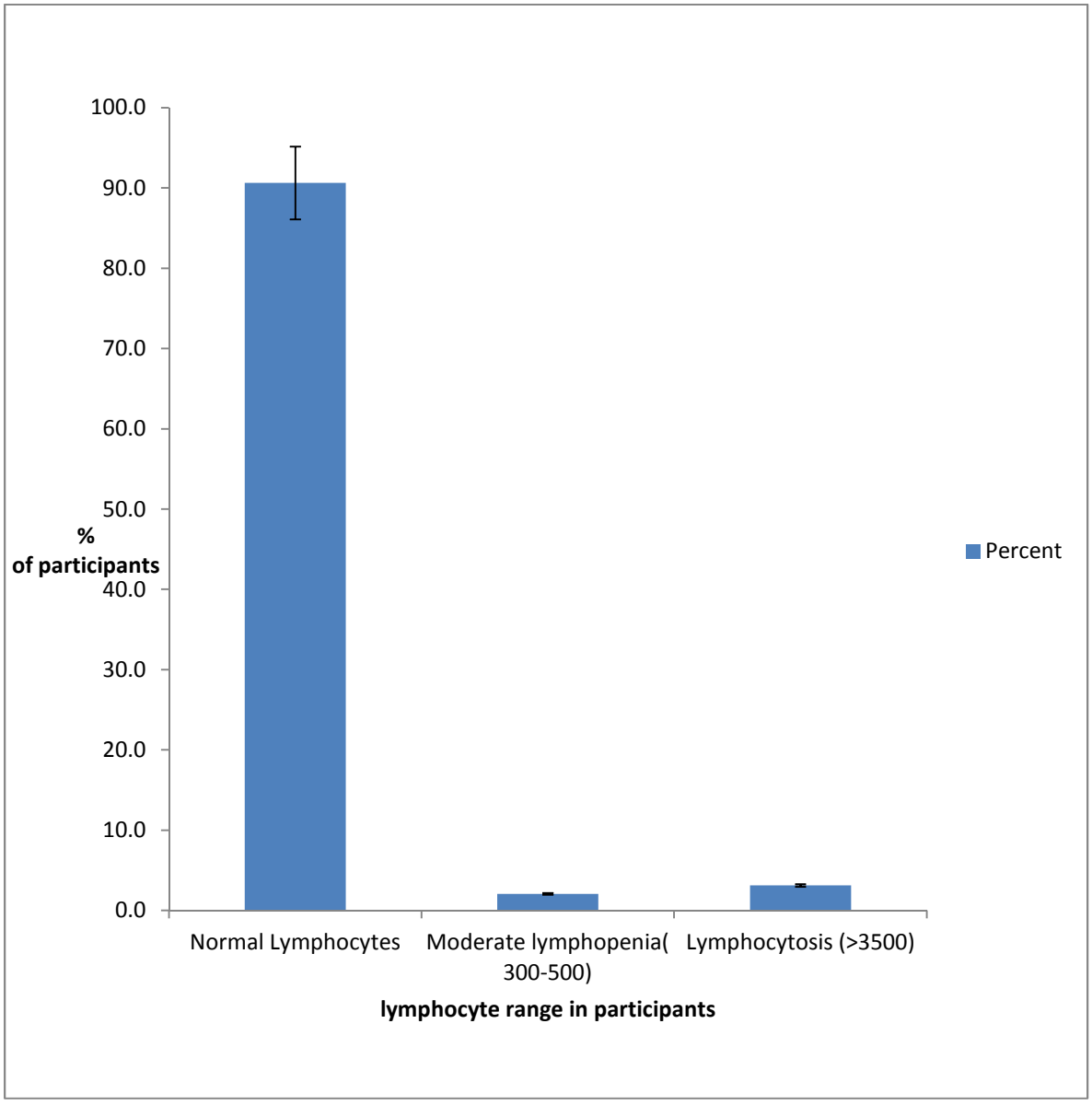

Figure 5 Prevalence of lymphopenia in participants

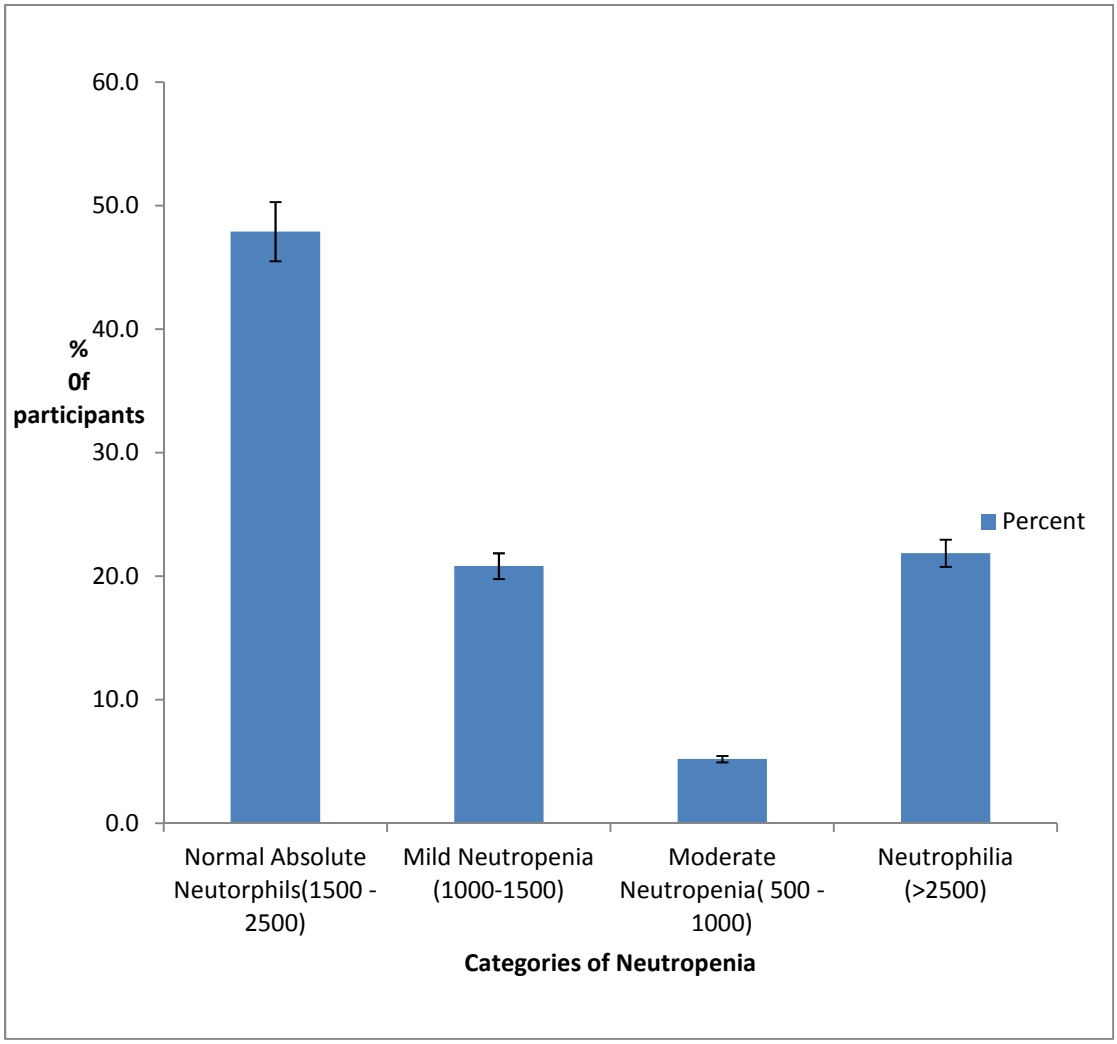

Figure 6 Prevalence of Neutropenia in participants

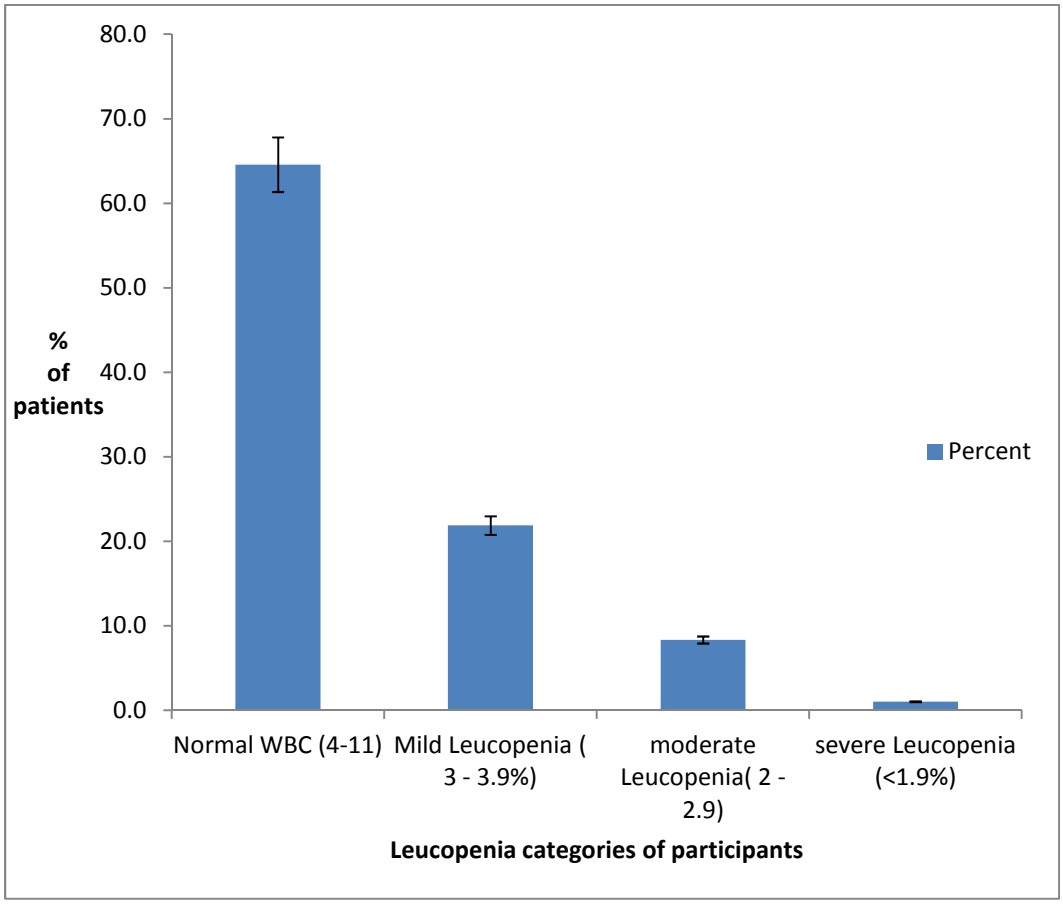

Figure 7 Prevalence of leucopenia in participants

Table 2 summary of immunological and hematological parameters

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Age	96	40.86	11.2	1.15
CD4(cells/µ/L) Baseline	96	395.81	273.05	27.89
RBC(million/µL/cu mm) Baseline	95	3.82	.76	.078
HB (g/dl) Baseline	96	12.00	1.81	.18
PCV (%) Baseline	96	36.39	4.10	.41
PLT(x 10^3/mL) Baseline	96	244.68	64.25	7.29
Absolute Lymphocyte count Baseline	96	2078.720	889.864	90.821
LYMP (%) Baseline	96	45.09	10.177	1.039
Absolute Neutrophil Count (Baseline)	96	1897.26	823.92	84.09
NUTR (%) Baseline	96	42.83	11.04	1.12
WBC(10^3/mm^3) Baseline	96	4.76	1.23	0.13
TNFα(pg/ml)	96	66.27	23.43	8.21

Table 3: Immunological and hematological differences observed in HIV patients in Rivers state.

	CD4 (P-value)	TNF-α	WBC
CD4> 500	689.76±205.104	43.11±23.459	5.44±1.41
CD4 200 - 500	363.06±88.116*(0.01)	62.25±49.748*(0.02)	4.76±1.17
CD4< 200	99.72±42.293**(<0.001)	64.06±68.255*(0.03)	4.15±1.29
	ALC	**ANC**	**LYMP (%)**
CD4> 500	2467.74±913.90	2189.14±908.73	47.76±8.88
CD4 200 - 500	2088.30±795.82	2003.29±965.58	45.21±9.81
CD4< 200	1624.81±770.62**(0.02)	1963.35±863.97*	41.93±11.36
	NUT (%)	**Hb**	**PLT**
CD4> 500	46.66±12.49	12.52±1.50	209.48±75.65
CD4 200 - 500	41.52±9.53	11.65±2.07	211.54±66.16
CD4< 200	40.85±10.61	11.83±1.71	239.79±86.86

N=96
Results expressed as Mean±SE(p-value)
"*" shows significant difference when compared to patients with CD4 <200
"" shows significant difference when compared to patients with CD4 between 200-500**

DISCUSSION

The demographics of participants are in table 1. Table 2 shows the distribution of CD4 counts in participants of the study, 34.4% of the patients had CD4 counts greater than 500, 35.4% was within 200-500 while 36.2% had CD4 count less than 200.

Fig 2 shows the severity of anemia in the population. About 56.3% of the population had a normal concentration of hemoglobin, only 1% of the participants had severe anemia, mild and moderate anemic subjects were 33.3% and 9.4% respectively.

Fig 3 shows the prevalence of thrombocytopenia, prevalence of thrombocytopenia was 11.5%, in this population, mild thrombocytopenia was present in 9.4% of the participants while severe thrombocytopenia was present in 2.1% of participants.

Prevalence of neutropenia was also estimated, prevalence was 26% amongst which 2.8% of participants were in the mild neutropenia group, 5.2% of the participants were in the moderate Neutropenia group, whereas, 21.9% presented with neutropenia.

Lymphopenia was also investigated. About 2.1% of the participants presented with lymphopenia whereas 3.1% had lymphocytosis

Leucopenia was accessed in participants using WBC, 21.9%of participants presented with mild leucopenia, 8.3% of participants had moderate leucopenia while 1% had severe leucopenia.

CD4 count and TNF α were immunological markers used to access the immunological system. The CD4 count of the participants was observed and results showed that the mean CD4 count was 395.81 ± 273.6 cells/μ/L, mean tumor necrosis factor alpha (TNF α) was 65.33 ± 58.53.

The Mean hemoglobin (Hb) concentration of participants was also accessed and was pegged as Hb, 12 ± 1.81g/dl. Packed cell volume (PCV) was 36.39 ± 4.1 %, while platelet (PLT) count was 245.00 ± 66.00 * 10^3/ml.

The relative lymphocyte count was 45 ± 10%, while the Absolute lymphocyte count was 1897.20 ± 10 823.92.

The relative neutrophil count neutrophil was 42.823± 11.05 %. Absolute neutrophil has 1897.21± 823.9. White blood cell count (WBC) was 4.82± 1. 382 $10^3/mm^3$.

3.2 Prevalence of Cytopenia in participants

In this study Anemia, leucopenia, neutropenia and thrombocytopenia were common findings. These same findings have been documented by [10, 11, and 12]. In this study prevalence of Anemia was 43.7%, which was higher thrower than the 65.5% prevalence found in India in 2008 [13]. Another study in Brazil recorded 37.5% [14]. Furthermore, these values were lower than that done in Ogun state of Nigeria, where prevalence was 74%. [15].

The report of this study is also consistent with those reported by [16, 17], where they stated that the prevalence of severe anemia declined while mild to moderate anemia remained common. The anemia may be as a result of serum erythropoietin levels, [18] auto-antibodies to erythropoietin, or marrow suppression by opportunistic infections, and medications [19].

A leucopenia prevalence of 30.3% was observed in this study, which was higher than the 26.6% recorded by [20], prevalence of neutropenia and lymphopenia in this study, which was 26% and 2.1% respectively and these findings were consistent with those of [20].

The findings in this study also show that the results were lower than prevalence found by [21].

It was also observed that the CD4 count of patients in this study was 395.81 ± 273.05; this reduced CD4 count may explain the high prevalence of leucopenia and neutropenia.

This may be as a result of increased suppression of bone marrow and direct T helper cell infection.

The prevalence of thrombocytopenia was 11.5% in this study and this was lower when compared to studies in Lagos [22] were prevalence was 1%. Thrombocytopenia may be due to destruction of platelets by immune cells [23].

The present study also observed lymphocytosis prevalence to be 3.1%, this is consistent with studies by [24, 25], they reported that physiologic lymphocytosis was peculiar to West Africans.

3.3 Immunological markers in patients.

The mean CD4 count of participants in this study was 395.81± 273. 05, this value was higher than that reported by [26] findings were higher than those reported by [27] in the UK, values were 270 cells.

Tumor necrosis factor plays a major role in HIV, TNF α levels have been found to be consistently high in HIV patients of African origin [28]. The present study observed TNF α levels to be 65.33±58.2pg/ml, this result were greater than those obtained from [28]. Serum level of TNF alpha was higher in patients with CD4 less than 200 as shown the results.

The increased serum levels of TNF-alpha are not paralleled by increased ability of immune cells to respond positively upon stimulation by inducing agents; rather this ability weakens with disease progression.

Since persistent TNF α activation may have an important pathogenic role, it will be useful to observe TNF α levels in HIV patients.

4.0 CONCLUSION

The most common hematological abnormalities in the study were anemia,

leucopenia and neutropenia, the prevalence of anemia was high, and prevalence of leucopenia and thrombocytopenia were also high. High levels of tumor necrosis factor alpha (TNF α levels) were also observed. The mean CD4 count was observed to be between 200-500 cells/ml. Patients with CD4 less than 200 showed increased TNF α, reduced CD4, total lymphocyte and neutrophil counts. Finding from this study has led to the recommendation that these parameters be used as biomarkers in monitoring patients and disease control in Nigeria. Therefore, patients should continuously monitor Absolute lymphocyte and neutrophil counts, TNF α levels, hematological and hematological dysfunctions regularly. These parameters are less expensive and readily available when compared to CD4 monitoring which is characterized with low or no availability in some rural areas, constant epileptic power problems, lack of reagents and lack of proper equipment maintenance.

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Conflict of Interest: The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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