



## Haematological Indices and Absolute CD4 Counts of Apparently Healthy Population in Ondo State, Nigeria

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### Authors' contributions

*This work was carried out in collaboration between all authors. Authors BDA and ADA designed and coordinated the study. Authors ADA and BDA performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HBF and BDA managed the analyses of the study. Author NOI assessed the participants clinically. Author HBF coordinated sample collection while authors NOI and ADA managed the literature searches. All authors read and approved the final manuscript.*

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### ABSTRACT

**Aims:** This study was designed and conducted to establish the normal values of various haematological indices and CD4 absolute count for apparently healthy adult in Ondo State, and to compare these values with those obtained for other populations in both tropical and western countries.

**Study Design:** Cross sectional study.

**Place and Duration of Study:** Department of Haematology and the global HIV/AIDS Initiative

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Nigeria (GHAIN), Federal Medical Centre (FMC), Owo. Nigeria between July 2013 and June 2014.

**Methodology:** Haematological indices and absolute CD4 count were determined in four hundred and eighty one (481) apparently healthy individuals whose ages ranged between 20–50 years, were randomly recruited into this study from the Haematology department, Federal Medical Centre, Owo.

**Results:** The mean blood levels of HCT, RBC, HB and MONO were significantly lower in female subjects compared with male subjects. Also, the mean blood levels of HCT, RBC and HGB were significantly lower while the mean blood level of Retics and ESR were significantly higher with decrease in age group. The comparative values for males and females in this study were also compared with previous established baseline values in Nigeria, Kenya and US.

**Conclusion:** This study was able to establish normal reference values for hematological indices and CD4 counts in apparently normal adults in Ondo State, Nigeria. This range could be useful as a basis for making some clinical decisions and also in enrolling HIV patients into the ART program when absolute CD4 counts are considered.

*Keywords: Haematological indices; absolute CD4 count; reference range; apparently healthy adults.*

## 1. INTRODUCTION

There is adequate information on haematological baseline, and this is varying depending on different population groups and geographical areas [1,2]. The variations might be as a result of environmental factors and social differences [3], so also age and gender had been documented to cause the variations too [2].

Genetic is another factor that had been established to contribute to all blood cell measures, accounting variation ranging between 61%–96% [4,5]. Several studies have been carried out on children and adolescents of various ages, and significant differences have been reported in different populations, seasons, racial and ethnic groups, and gender subgroups [5,6]. In most of the studies conducted so far in Nigeria, significant differences in normal laboratory ranges have been reported compared with those of other African countries and industrialized world [7] which could be due to a number of reasons including differences in geographical locations, climate, dietary habits, and environmental factors or ethnic and tribal peculiarities. Owing to this, it has therefore been stressed that each population must establish its own “normal reference values” for use in clinical assessments [5,7]. Therefore, this justifies why it is necessary to establish baseline haemogram values specific for indigenous populations of different locality in Nigerian populace.

Knowledge of the CD4 count in an individual is of immense value in the evaluation of body immune system [8]. The CD4+ T lymphocytes are the crucial cells in the cascade of events in forming immune response to the foreign antigen and

hence monitoring the CD4+ T cell counts are necessary to understand the extent of immune deficiency [8,9]. However, haematological reference values and CD4 T lymphocytes among apparently healthy adults have been established in many populations of Africans including Nigerians [7], yet there is a dearth of information regarding hematological and CD4 normal reference values for adults in Ondo State, Nigeria.

Medical laboratory tests are also very important tool for diagnosing health disorders, disease staging and monitoring of response to treatment. Interpretation of laboratory test results appropriately requires standard reference values and it helps in providing quality services in the Health care delivery. Inability to provide standard local reference values for all regions has been a problem facing hematological practice in Nigeria. This study, thus, aimed at bridging the gap in apparently normal adults in our locality for future use in Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Subjects

A cross sectional study of the haematological parameters of apparently healthy adults was carried out over a period of one year, between July 2013 and June 2014 at Federal Medical Centre (FMC), Owo. This hospital is a reference centre which provides general and specialized quality services in the Health care delivery for the people of Ondo State. After obtaining an approval from the Federal Medical Centre (FMC) Joint Ethics Review Committee (FMC/EC/102014) and written informed consent (approved

by the FMC Ethics committee) from each subject, a total of four hundred and eighty one (481) apparently healthy individuals were randomly recruited into this study from the haematology department, Federal Medical Centre, Owo.

## 2.2 Inclusion Criteria

Apparently healthy volunteers who has not been on medication for the last one month and age between 20-50 years. This was established by a careful medical history, designed structured questionnaires and complete physical examination. Seronegatives for both Human immunodeficiency virus (HIV) and Hepatitis B surface antigen (HBsAg) and non-reactive for Veneral disease of research laboratory (VDRL) were selected for the study.

## 2.3 Exclusion Criteria

Subjects with genotype HbSS, diabetes mellitus, hypertension, human immunodeficiency virus (HIV), hepatitis, cancer, regular smokers and individuals who had vaccination during the past six months were excluded from this study. Subjects with recent history of blood loss or who have received any blood transfusions in last 12 Months were also excluded from the study. Additionally, women on oral contraceptive or menstruating, pregnant and lactating mothers were also excluded from the study.

## 2.4 Blood Collection

Five (5) milliliters of blood sample was collected into ethylene diamine - tetra acetic acid (EDTA) vacutainer tubes for CD4 and complete blood count between 9.00 am to 12.00 noon with all bio-safety precautions [9]. Blood samples were transported in cold chain boxes to the global HIV/AIDS Initiative Nigeria (GHAIN) for CD4 absolute counts and haematology assays, done within six hours of sample collection.

## 2.5 Haematology Assay

Blood parameters including packed cell volume (PCV), white blood cell (WBC), MCV, MCH and MCHC counts were determined using the Automated Haematologic Analyzer, Sysmex, KX-N21 (Japan) as described by Olaniyi et al. [10] and Akinbo et al. [11]. Erythrocyte sedimentation rate was done by Westergreen method [5].

## 2.6 Immunologic Indices Analysis

The samples were assayed using the Partec cyflow counter. Flow cytometry (cell measurement) is a process used to count, identify, and sort various types of cells. This technique is based on adding monoclonal antibodies (MAb) to a blood sample and running the fluid through a light source, usually a laser beam [8,12,13].

## 2.7 Statistical Analysis

Reference ranges were calculated using non-parametric methods. The medians were calculated and reference values were determined at 2.5<sup>th</sup> and 95<sup>th</sup> percentiles. Mean, median and standard deviations were computed for each of the clinical hematological parameters and CD4 counts of the study subjects. Parametric student's t-test was used to determine any statistically significant differences between males and females. Analysis of variance (ANOVA) was used to compare the differences in mean age group.  $P < 0.05$  was considered significant. All statistical analyses were done using SPSS version 17.0.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

Out of five hundred and four (504) participants that were initially recruited for this study, 14 (2.8%) participants had low haematocrit and haemoglobin were excluded from the study, also 9 (1.8%) participants were excluded due to CD4 counts outside of established normal value for the country by other studies [7] and 481 (95.4%) participants with full data were recruited for the study. Mean standard deviation (SD), median and baseline values for the various haematological indices and CD4 absolute count are presented in Tables 1 and 2 respectively.

In Table 3, the mean blood levels of HCT, RBC, HB and MONO were significantly lower in female subjects compared with male subjects. In Table 4, the mean blood levels of HCT, RBC and HGB were significantly lower while the mean blood level of Retic and ESR were significantly higher with decrease in age group. The comparative values for males and females in this study with previous established baseline values in Nigeria, Kenya and USA are presented in Table 5.

**Table 1. Haematological indices of apparently healthy adult in Ondo State**

Subjects	Indices	WBC (x10 <sup>3</sup> /UL)	RBC (x10 <sup>6</sup> /UL)	HB (g/dl)	HCT (%)	Retics (%)	PLT (x10 <sup>3</sup> /UL)	LYM (%)	NEUT (%)	MONO (%)	BASO (%)	EOSIN (%)	ESR (mmHg)
Male (n=222)	Mean±SD	6.53±1.67	4.61±0.40	13.8±1.40	40.9±4.0	0.80±0.51	195.6±43.8	35.4±5.4	62.3±5.8	1.2±1.5	0.1±0.3	1.0±1.3	2.2±1.1
	Ref range	4.2-9.7	4.0-5.3	11.6-16.3	35.0-48.0	0.2-1.8	152.0-288.0	25.0-43.0	50.0-71.0	0.0-4.0	0.0-1.0	0.0-4.0	0.0-4.0
	Median	6.30	4.55	13.70	43.6	0.60	299.6	36.0	62.0	2.60	0.00	0.00	2.00
Female (n=259)	Mean±SD	6.40±1.70	4.34±0.33	13.1±1.1	39.0±3.0	1.10±0.50	199.2±43.7	34.3±5.5	63.3±5.9	1.1±1.3	0.1±0.3	1.2±1.4	4.7±1.2
	Ref range	4.2-9.6	3.9-5.0	11.3-15.3	35.0-45.0	0.2-1.8	151.0-302.0	24.0-42.0	52.0-72.0	0.0-4.0	0.0-1.0	0.0-4.0	2.0-6.0
	Median	6.00	4.30	12.80	39.0	1.20	187.0	34.0	64.0	1.00	0.00	1.00	5.00

Key: SD=Standard Deviation, WBC=White Blood Cells, RBC=Red Blood Cells, HB=Haemoglobin, PLT=Platelets, LYM=Lymphocyte, NEUT=Neutrophil, MONO=Monocyte, BASO=Basophil, EOSIN=Eosinophil, ESR=Erythrocyte Sedimentation Rate, n=Sample size

**Table 2. CD4 absolute count of apparently healthy adults in Ondo State**

Sex	N	Mean± SD	Median	Range	2.5 <sup>th</sup> -95 <sup>th</sup> percentile
Combined	481	765.8±243.5	738.0	403.0-1900.0	424.0-1288.0
Male	222	765.2±236.7	753.0	404.0-1650.0	420.5-1265.0
Female	259	766.3±249.6	716.0	403.0-1900	426.5-1300.0

**Table 3. Comparative haematological indices and CD4 absolute count for Male with Female in apparently healthy adults of in Ondo State**

Subjects (n)	Indices	WBC (x10 <sup>3</sup> /UL)	RBC (x10 <sup>6</sup> /UL)	HB (g/dl)	HCT (%)	Retics (%)	PLT (x10 <sup>3</sup> /UL)	LYM (%)	NEUT (%)	MONO (%)	BASO (%)	EOSIN (%)	ESR (mmHg)	CD4 (Cells/ml)
Male (n=222)	Mean±SD	6.53±1.67	4.61±0.40	13.8±1.40	40.9±4.0	0.80±0.51	195.6±43.8	35.4±5.4	62.3±5.8	1.2±1.5	0.1±0.3	1.0±1.3	2.2±1.1	765.2±236.7
Female (n=259)	Mean±SD	6.40±1.70	4.34±0.33	13.1±1.1	39.0±3.0	1.10±0.50	199.2±43.7	34.3±5.5	63.3±5.9	1.1±1.3	0.1±0.3	1.2±1.4	4.7±1.2	766.3±249.6
	P-value	0.387	0.000	0.000	0.000	0.056	0.614	0.300	0.427	0.019	0.901	0.453	0.131	0.541

Significant (p<0.001), Key: CD4=Cluster of differentiation

**Table 4. Comparative haematological indices and CD4 absolute count between different age group in apparently healthy adults in Ondo State**

Age Group (Years)	Indices	WBC (x10 <sup>3</sup> /UL)	RBC (x10 <sup>6</sup> /UL)	HB (g/dl)	HCT (%)	Retics (%)	PLT (x10 <sup>3</sup> /UL)	LYM (%)	NEUT (%)	MONO (%)	BASO (%)	EOSIN (%)	ESR (mmHg)	CD4 (Cells/ml)
20-29 (n=162)	Mean±SD	6.31±1.61	4.32±0.33	13.0±1.1	38.5±2.8	1.18±0.48	198.8±47.8	35.1±5.8	62.4±6.2	1.2±1.5	0.1±0.3	1.2±1.6	4.2±1.6	770.3±259.6
30-39 (n=200)	Mean±SD	6.60±1.87	4.45±0.34	13.3±1.1	39.7±3.4	1.0±0.48	199.4±43.8	34.4±5.4	63.3±5.8	1.0±1.3	0.1±0.3	1.1±1.2	3.5±1.6	748.1±224.5
40-49 (n=119)	Mean±SD	6.36±1.55	4.69±0.43	14.1±1.5	42.0±3.9	0.70±0.5	192.7±37.3	34.9±5.1	62.7±5.5	1.2±1.4	0.1±0.3	1.1±1.2	2.8±1.7	789.5±251.3
	P-value	0.228	0.000	0.000	0.000	0.000	0.382	0.522	0.291	0.325	0.932	0.556	0.000	0.327

Significant (p<0.001)

**Table 5. Haematological baseline values comparisons with other established reference range**

Sex	Indices	Ondo State (This study)	Nigeria [7]	Kenya [14]	USA [15]
Males	WBC (x10 <sup>3</sup> /UL)	4.2-9.7	4.3-4.6	2.8-8.2	4.5-11.0
	RBC (x10 <sup>6</sup> /UL)	4.0-5.3	5.1-5.3	4.4-6.3	4.5-5.9
	HB (g/dl)	11.6-16.3	14.0-14.4	8.3-11.3	13.5-16.0
	HCT (%)	35.0-48.0	43.5-45.0	40-50	41-53
	Retics (%)	0.2-1.8	N/A	N/A	N/A
	PLT (x10 <sup>3</sup> /UL)	152.0-288.0	206.8-226.8	120-411	150-350
	LYM (%)	25.0-43.0	37.4-40.2	20-60	22-44
	NEUT (%)	50.0-71.0	52.6-55.2	40-60	40-70
	MON0 (%)	0.0-4.0	5.3-6.4	3-11	4-11
	BAS0 (%)	0.0-1.0	0.02-0.12	0-2	0-3
	EOSIN (%)	0.0-4.0	1.1-1.9	1-20	0-8
	ESR (mmHg)	0.0-4.0	N/A	N/A	N/A
CD4 (Cells/ml)	420.5-1265.0	314-1347	N/A	N/A	
Females	WBC (x10 <sup>3</sup> /UL)	4.2-9.6	4.4-4.8	N/A	N/A
	RBC (x10 <sup>6</sup> /UL)	3.9-5.0	4.5-5.3	3.7-5.6	4.0-5.2
	HB (g/dl)	11.3-15.3	12.4-13.1	5.9-10.0	12.0-16.0
	HCT (%)	35.0-45.0	38.8-40.5	30-50	36-46
	Retics (%)	0.2-1.8	N/A	N/A	N/A
	PLT (x10 <sup>3</sup> /UL)	151.0-302.0	229.3-251.2	N/A	N/A
	LYM (%)	24.0-42.0	39.0-42.1	N/A	N/A
	NEUT (%)	52.0-72.0	49.1-52.3	N/A	N/A
	MON0 (%)	0.0-4.0	6.5-7.5	N/A	N/A
	BAS0 (%)	0.0-1.0	0.06-0.18	N/A	N/A
	EOSIN (%)	0.0-4.0	1.3-1.9	N/A	N/A
	ESR (mmHg)	2.0-6.0	N/A	N/A	N/A
CD4 (Cells/ml)	426.5-1300.0	358-1328	N/A	N/A	

**3.2 Discussion**

Most of reference values of hematologic parameters currently used in Nigeria and Africa at large are derived from data collected for populations living in industrialized countries. In most of the studies conducted so far in Nigeria, significant differences in normal laboratory ranges have been reported compared with those of other African countries and industrialized world [7] which could be due to a number of reasons including differences in geographical locations, climate, dietary habits, and environmental factors or ethnic and tribal peculiarities.

The observed values of hematological indices in this study were slightly significant different from standard reference values from United States (US). Similar differences were also observed when the normal baseline range of this study was compared to values of other studies done in Nigeria, African populations [7]. The difference in findings of this study and those reported elsewhere could be due to some reasons listed above [2,5,6].

The significant differences are more obvious among the female groups when compared to male group counterparts. The females showed a marked lower Haemoglobin concentration, Haematocrit values and red blood cells (RBC) than the males; this confirmed previous study that gender could also cause the variations too and this may be due to the variations in hormone types and the effect of erythropoietin release in response to regular menstruation [2,7]. Several differences were observed when compared to previously established values from both local and western world. Most notably in hemoglobin, packed cell volume, platelets, total white blood cell count, and neutrophil values which might be due to geographical areas, climate, dietary habits, and environmental factors or genetical variations [1,2].

The CD4 T-lymphocyte absolute count is one of the best surrogate markers for assessing the risk of progression to AIDS among HIV-infected individuals. This marker is also a diagnostic tool in determining the risk of developing certain AIDS related opportunistic infections or immune compromised conditions and the time for initiating antiretroviral and prophylactic

antimicrobial therapies [16,17]. From the results of this study, it is concluded that the baseline absolute CD4 T cell count at 2.5th- 95th percentile reference intervals for females and males are 426.5-1300.0 cells/ml and 420.5-1265.0 cells/ml respectively. Females subjects in this study had higher CD4 counts than males as earlier described by other investigators [7,17], which might be as a result of haemopoietic feedback stimulus following monthly blood loss due to regular menstruation. The age group 30-39 years has a significantly lower CD4 absolute count comparing to other age groups. Since some factors had been established to associate with low CD4 cell counts, including psychological stress [17], it is plausible that the young population about to marry or new married population undergo more stress than the unmarried and those that had married for long time counterparts, resulting in the observed trend.

#### 4. CONCLUSION

In conclusion, this study, therefore, was able to establish normal reference values for hematological indices and CD4 counts in apparently normal adults in Ondo State, Nigeria. This range could be useful as a basis for making some clinical decisions and also in enrolling HIV patients into the ART program when absolute CD4 counts are considered. Furthermore, significant difference values observed in this study as shown in above Table 5 justified why it is necessary to establish baseline haemogram values specific for indigenous populations of different locality in Nigerian populace.

#### CONSENT

All authors declare that written informed consent was obtained from each subject before being enrolled into the study.

#### ETHICAL APPROVAL

This study was approved by the Federal Medical Centre (FMC) Joint Ethics Review Committee. Also, written informed consent (approved by the FMC Ethics Committee) was obtained from each subject.

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(GHAIN) and department of haematology, FMC, Owo, Nigeria.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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