



ORIGINAL RESEARCH

Leucocyte Profiles of Asymptomatic and Symptomatic HIV-1 Subjects in Irrua, EDO State, Nigeria

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Abstract:

HIV is pandemic and remains a public health concern for many decades. This infection is associated with life threatening opportunistic infections and malignancies. The aim of this study is to look at the leucocyte profiles of HIV-1 positive subjects in Irrua, Edo state, Nigeria. A total of three hundred and forty four (344) HIV-1 subjects aged 17-60 years and of both sexes were recruited into the study. The HIV-1 subjects were divided into asymptomatic and symptomatic subjects. One hundred and fifty (150) apparently healthy HIV-1 negative subjects served as control. The white blood cells total (WBC total) count and differential leucocyte count (DLC) were determined using an automated blood analyzer (Sysmex KX-2IN). Results obtained revealed that the mean WBC total count ($\times 10^3/\mu\text{L}$) of the asymptomatic HIV-1 subjects (5.04 ± 1.14) and that of the control subjects (5.09 ± 1.24) did not show any significant difference ($P > 0.05$). In contrast, the mean WBC total count of symptomatic HIV-1 subjects (4.14 ± 5.06) was significantly decreased ($P < 0.05$) in comparison to that of control. However, age and sex did not affect the WBC total counts of the subjects studied. With respect to DLC, the mean neutrophil % of asymptomatic HIV-1 subjects (43.53 ± 8.84) was not statistically significant ($P > 0.05$) in comparison to control (43.91 ± 5.91). Whereas, symptomatic HIV-1 subjects (49.25 ± 5.86) revealed a significantly higher ($P < 0.05$) difference in relation to control (43.91 ± 6.25) and asymptomatic HIV-1 subjects. The mean lymphocyte count % of asymptomatic HIV-1 subjects (42.19 ± 6.25) and that of symptomatic HIV-1 subjects (43.32 ± 6.92) were significantly lower ($P < 0.05$) compared to control (47.11 ± 8.33). Age and sex also did not affect the DLC of the study subjects. In conclusion, it has been revealed that the leucocyte profile of symptomatic HIV-1 subjects were significantly affected compared to asymptomatic and control subjects. It re-emphasizes the prognostic role of leucocyte counts (especially in low resource areas) in the diagnosis of symptomatic HIV-1 subjects in the study area.

Key words: Leucocytes, HIV-1, Symptomatic subjects, Asymptomatic subjects, Irrua.

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Introduction:

Acquired immune deficiency syndrome (AIDS) is a disease of the human immune system caused by the Human Immunodeficiency Virus (HIV). HIV is a lentivirus, a member of the retrovirus family and its infection can lead to AIDS, a condition in which the immune system begins to fall leading to life threatening opportunistic infection (Phillip *et al.*, 1996). Sub-Saharan Africa (divided now in UNAIDS reports into Eastern/Southern Africa and Western/Central Africa) continues to bear the brunt of this pandemic with 25.5 million adults and children (69% of global total) living with HIV in 2015 (UNAIDS, 2016). In Nigeria, NAIIS (2018) has revealed a national HIV prevalence of 1.5% for adults aged 15-64 years with the estimated population of people living with HIV aged 0-64 years being 1.9 million.

White blood cells also called leukocytes or leucocytes and abbreviated as WBCs are the cells of the immune systems that are involved in protecting the body against infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as haemopoietic stem cells (Maton *et al.*, 1997). Two pairs of broadest categories classify them either by structure (granulocytes or agranulocytes) or cell lineage (myeloid cells or lymphoid cells). These broad categories can be further divided into five main types: Neutrophils, eosinophils (acidophiles), basophils, lymphocytes and monocytes (LaFleur-Brooks, 2008). White blood cells make up 1% of the total blood volume of a healthy adult (Alberts *et al.*, 2002) making them substantially less numerous than the red blood cells at 40-45%. However, this 1% of the blood makes a large difference to health because immunity depends on it. The present study was therefore aimed at investigating the leucocyte profiles of asymptomatic and symptomatic HIV-1 subjects in our locality.

Materials and Methods:

A total of three and forty four (344) HIV-1 subjects which comprised of two hundred (200)

asymptomatic HIV-1 subjects (group 1) and one hundred and forty four (144) symptomatic HIV-1 subjects (group 2) were recruited for this study. One hundred and fifty (150) apparently healthy HIV-1 negative subjects served as control (groups 3). The subjects were between 17 and 60 years of age and of both sexes. All the categories of subjects were randomly and voluntarily recruited after informed consent had been sought. Selection of all the categories of subjects recruited for this study were based on inclusion and exclusion criteria and clinical stage of the subjects based on WHO clinical staging of HIV/AIDS and case definition for resource-constrained settings. Ethical clearance was obtained from the Research and Ethics Committee of Irrua Specialist Teaching Hospital, Irrua, Edo State.

Blood collection - Four millilitres (4ml) of whole blood was withdrawn by venipuncture from the antecubital vein using vacutainer needles into EDTA containers. All the blood samples were collected during the day with all bio-safety precautions observed. All the investigations were carried out with minimal delay.

Haematology Assay -White blood cells counts (that is total and differential leucocyte counts) were determined using automated haematology analyzer, Sysmex KX-2IN (Sysmex Corporation, Kobe, Japan). The Sysmex KX-2IN is an automatic, 19-parameters, 3-part differential blood cell counter. The procedure was carried out according to the manufacturer's instructions. The principle of this method is based on the DC (Direct Current) detection method.

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Statistical analysis - Experimental variables between two groups of individuals were analyzed using unpaired t-test. One-way ANOVA with Post hoc Dunn's multiple comparison tests (non-parametric) was used to analyze three or more groups. $P < 0.05$ was considered significant. Descriptive methods such as tables and figures were used for the presentation of the results.

Results:

The white blood cells (WBC) total counts of the subjects studied are presented in Table 1. The mean values of the WBC total counts of the control, asymptomatic and symptomatic subjects were 5.07 ± 1.24 , 5.04 ± 1.14 and 4.14 ± 0.56 respectively. Statistical comparison showed that there was no statistical significant difference ($p > 0.05$) between the asymptomatic subjects and control. However, comparison between the mean values of the symptomatic subjects and the control group showed a statistically significant difference ($P < 0.05$). The mean value of the differential leucocyte count (Figure 1) of the control subjects with respect to neutrophils % was 43.91 ± 5.91 against 43.53 ± 8.84 and 49.25 ± 5.86 for asymptomatic and symptomatic subjects respectively. There was no significant difference ($P > 0.05$) between the neutrophil % count of asymptomatic subjects and control. However, symptomatic subjects showed a significant increase ($P < 0.05$). Similarly, the mean values of lymphocytes % among the three groups (control, asymptomatic and symptomatic subjects) were 47.11 ± 8.33 , 42.19 ± 6.25 and 43.32 ± 6.92 respectively. Statistical comparison showed a significant decrease ($P < 0.05$) between the asymptomatic and symptomatic subjects in comparison to control. The middle cell count (MXD) % of the three groups were 8.98 ± 3.45 , 14.28 ± 5.08 and 7.43 ± 5.02 respectively. The control versus symptomatic subjects did not show any significant difference ($P > 0.05$). However, the asymptomatic subjects revealed a significant difference ($P > 0.05$).

The mean values of the WBC total count of the male and female subjects were 5.07 ± 0.91 and 5.03 ± 1.19 respectively (Table 2). There was no

statistical difference ($P > 0.05$) between both sexes. The WBC differential counts of asymptomatic subjects based on sex is shown in figure 2. The mean neutrophil % counts of the male and female subjects studied were 45.81 ± 6.61 and 42.99 ± 5.43 respectively. There was also no significant difference ($P > 0.05$) between the two sexes. In addition, the mean lymphocyte % counts of the male and female subjects were 41.23 ± 7.35 and 42.68 ± 5.65 respectively. Also statistical comparison did not show any significant difference between the two sexes. The mean value of middle cells MXD % between the male and female subjects studied were 12.96 ± 2.32 and 14.33 ± 4.33 respectively. Similarly, there was also no significant difference between the sexes.

The results of the mean values of the WBC total count according to the age groups of 17-26 years, 27-36 years, 37-46 years and 47 years and above were 5.06 ± 1.37 , 5.04 ± 1.27 , 5.01 ± 0.92 and 5.21 ± 0.98 respectively (Table 3). There was no statistically significant difference ($P > 0.05$) among the age groups. The WBC differential counts of asymptomatic subjects based on age was revealed in figure 3. The mean neutrophil count % according to the age ranges of 17-26 years, 27-36 years, 37-46 years and 47 years and above were 45.07 ± 6.24 , 42.61 ± 5.76 , 44.91 ± 7.02 and 42.64 ± 4.19 respectively. Similarly, the mean lymphocyte count % based on the age ranges of 17-26 years, 27-36 years, 37-46 years and 47 years and above were 39.01 ± 6.66 , 44.13 ± 6.73 , 42.23 ± 5.59 and 42.74 ± 5.50 respectively. Both the neutrophils % and lymphocyte % counts did not show any significant difference ($P > 0.05$) among the four age groups studied. Furthermore, the mean of the middle cells of the study subjects in the various age ranges of 17-26 years, 27-36 years, 37-46 years and 47 and above were 15.92 ± 3.14 , 13.26 ± 4.19 , 12.86 ± 3.34 and 14.62 ± 4.33 respectively. There was a statistically significant increase among subjects in the age range of 17-26 years compared to the remaining groups which did not show any significant difference ($P > 0.05$). Table 4 shows the WBC total count of symptomatic subjects based on gender. The mean values of the WBC total count of the male subjects was 4.32 ± 0.52 in comparison to

4.04 ± 0.57 for the female subjects. Statistical comparison did not show any significance (P>0.05).

The WBC differential count of symptomatic HIV-1 subjects based on gender is shown in Figure 4. The mean of neutrophil count % of the male and female HIV-1 subjects were 49.52 ± 6.71 and 49.13 ± 5.46 respectively. There was no significant difference between both sexes in relation to neutrophil count %. The mean lymphocyte count % of the male and female subjects studied were 40.72 ± 7.38 and 44.57 ± 6.45 respectively. There was also no significant difference (P > 0.05) between both sexes in relation to lymphocyte count %. The mean middle cells count % of 9.76 ± 3.20 and 6.30 ± 2.73 was recorded for the male and female subjects respectively. Similarly, there was no significant difference (P > 0.05) between both sexes in relation to middle cells %.

Table 5 shows the WBC total count of symptomatic subjects based on age. The WBC total count of the

study subjects according to the age groups of 17-26 years, 27-36 years, 37-46 years and 47 years and above were 3.90±0.42, 4.00±0.55, 4.23±0.64 and 4.21 ± 0.53 respectively. There was no significant difference among the various age groups. Figure 5 summarizes the WBC differential count of symptomatic HIV-1 subjects based on age. The neutrophils % of HIV-1 subjects belonging to the age groups of 17-26 years, 27-36 years 37-46 years and 47 years and above recorded mean values of 49.64±4.81, 47.13±6.30, 50.06±5.11 and 50.55±7.20 respectively. In a similar fashion, the mean values of 42.05±6.77, 44.83±6.58, 43.24±6.79 and 43.32±6.92 were also recorded for lymphocytes % across the various age groups. Furthermore, the middle cells (MXD) % had mean values of 8.31±2.18, 8.04±2.14, 6.70±2.04 and 6.13±2.01 against the respective age groups of 17-26 years, 27-36 years, 37-46 years and 47 years and above. Neutrophils %, lymphocytes % and middle cells % did not show any significant difference (P>0.05) in relation to age groups.

Table 1: WBC total count of the study subjects

Parameter	Control subjects Mean ± SD n = 150	Asymptomatic subjects Mean ± SD n = 200	Symptomatic subjects Mean ± SD n = 144	F-Value	P-Value
WBC TOTAL (x10 ³ /μL)	5.07 ± 1.24 ^a	5.04 ± 1.14 ^a	4.14 ± 0.56 ^b	20.910	0.000

Keys:

WBC = White blood cells

SD = Standard Deviation

n = Number of Samples

Values in a row with a different superscript is significantly different at P < 0.05

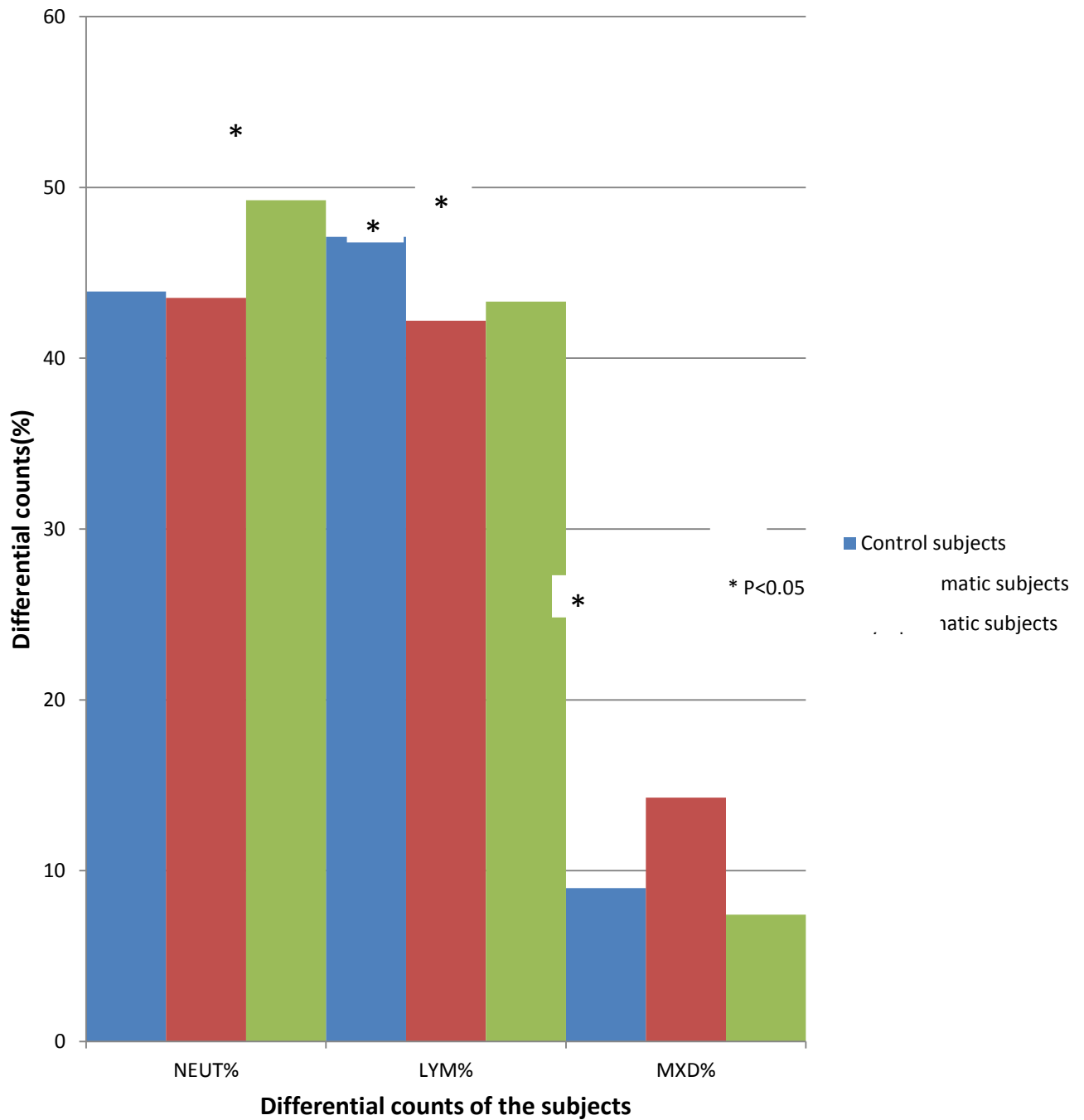


Figure 1: Differential leucocytes counts of the study population

KEYS:

NEUT % - Ratio (%) of neutrophils to whole WBC

LYM% - Ratio (%) of lymphocytes to whole WBC

MXD% - Ratio (%) of the summation of basophils, eosinophils and monocytes to whole WBC

*P<0.05 – values are significant at p<0.05

Table 2: WBC total count of asymptomatic HIV-1 subjects based on gender

Parameter	Male subjects Mean ± SD n = 80	Female subjects Mean ± SD n = 120	T-value	P-value
WBC total	5.07 ± 0.91	5.03 ± 1.19	1.187	0.062

KEYS:

- n - Number of Samples
- SD - Standard deviation
- WBC Total - WBC count in 1µL of whole blood

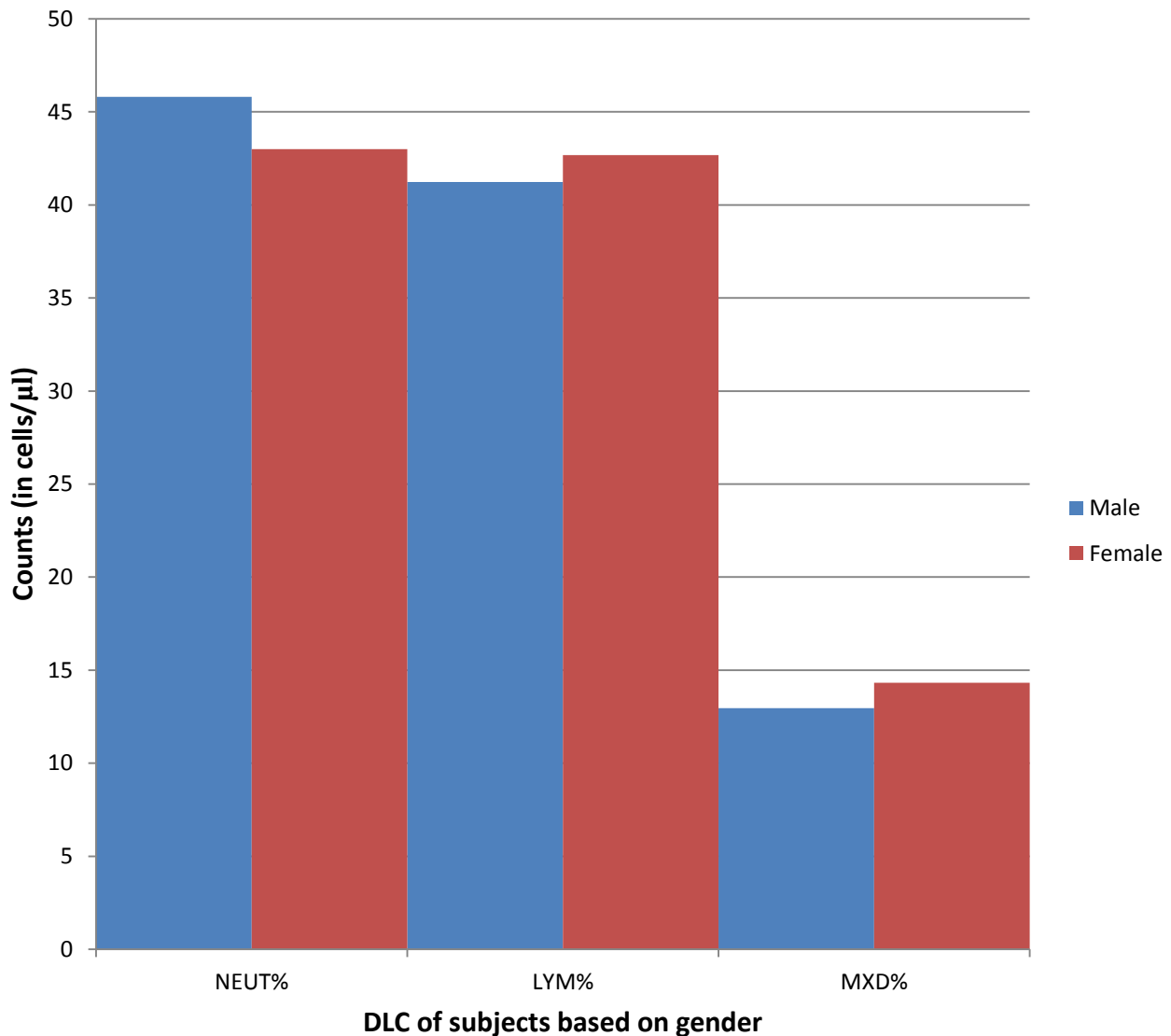


Figure 2: Differential leucocyte counts (DLC) of asymptomatic HIV – 1 subjects based on gender

KEYS:

- NEUT % - Neutrophil %
- LYM% - Lymphocyte %
- MXD% - Middle cells %

Table 3: WBC total count of asymptomatic HIV-1 subjects based on age

Parameter	17-26yrs Mean ± SD n = 14	27-36yrs Mean ± SD n = 60	37-46yrs Mean ± SD n = 100	47yrs & above Mean ± SD n = 26	F – Value	P – Value
WBC total	5.06 ± 1.37 ^a	5.04 ± 1.27 ^a	5.01 ± 0.92 ^a	5.21 ± 0.98 ^a	1.286	0.287

KEYS

WBC Total - WBC count in 1µL of whole blood

n - Number of Samples

SD - Standard deviation

Values in rows with different superscripts is significantly different at P<0.05

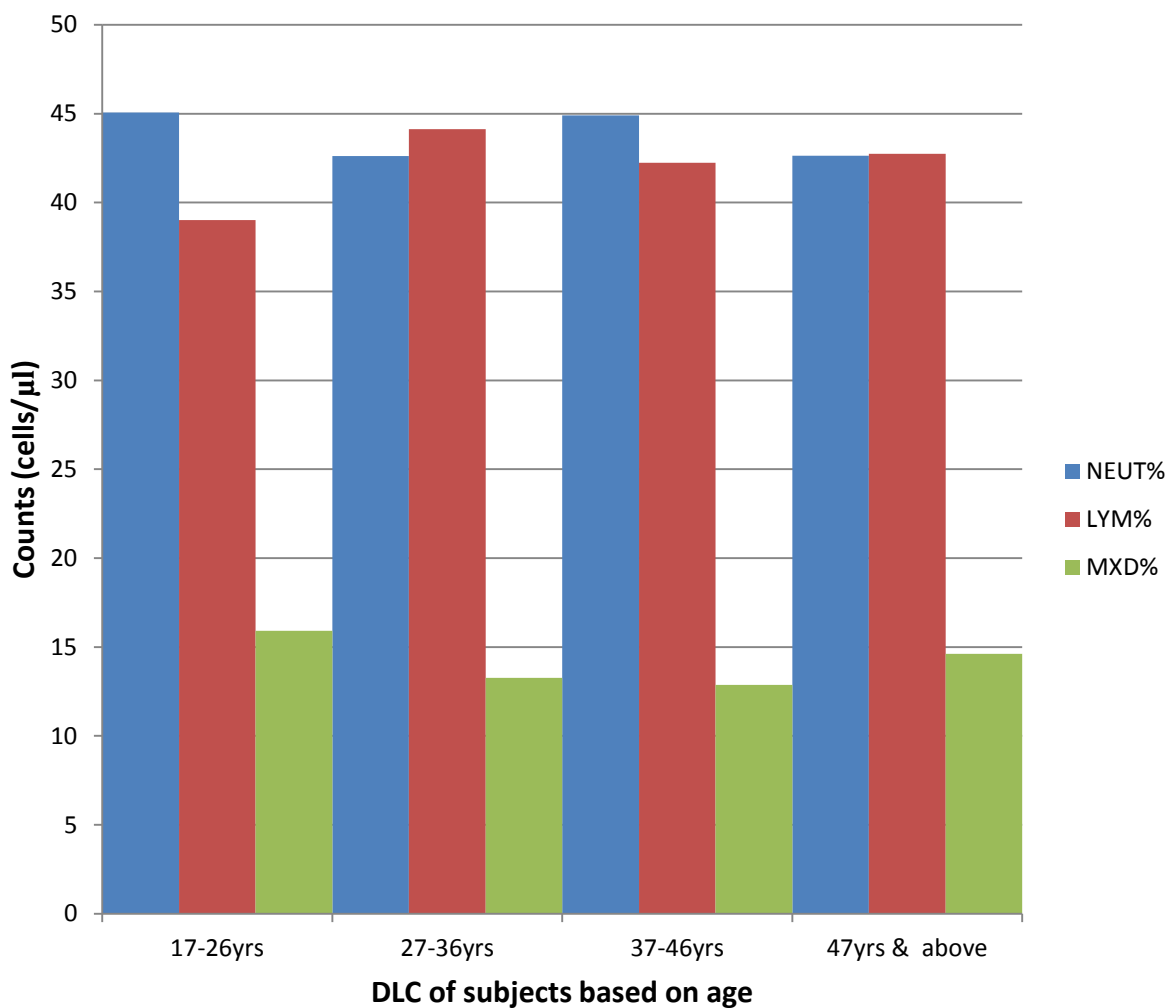


Figure 3: Differential leucocyte counts (DLC) of symptomatic subjects based on age

KEYS:

NEUT % - Neutrophil %

LYM% - Lymphocyte %

MXD% - Middle cells %

Table 4: WBC total count of symptomatic HIV-1 subjects based on gender

Parameter	Male Subjects Mean ± SD n = 58	Female subjects Mean ± SD n = 86	T-value	P-value
WBC total	4.32 ± 0.52	4.04 ± 0.52	1.487	0.145

KEYS:

- n - Number of Samples
- SD - Standard deviation
- WBC Total - WBC count in 1µL of whole blood

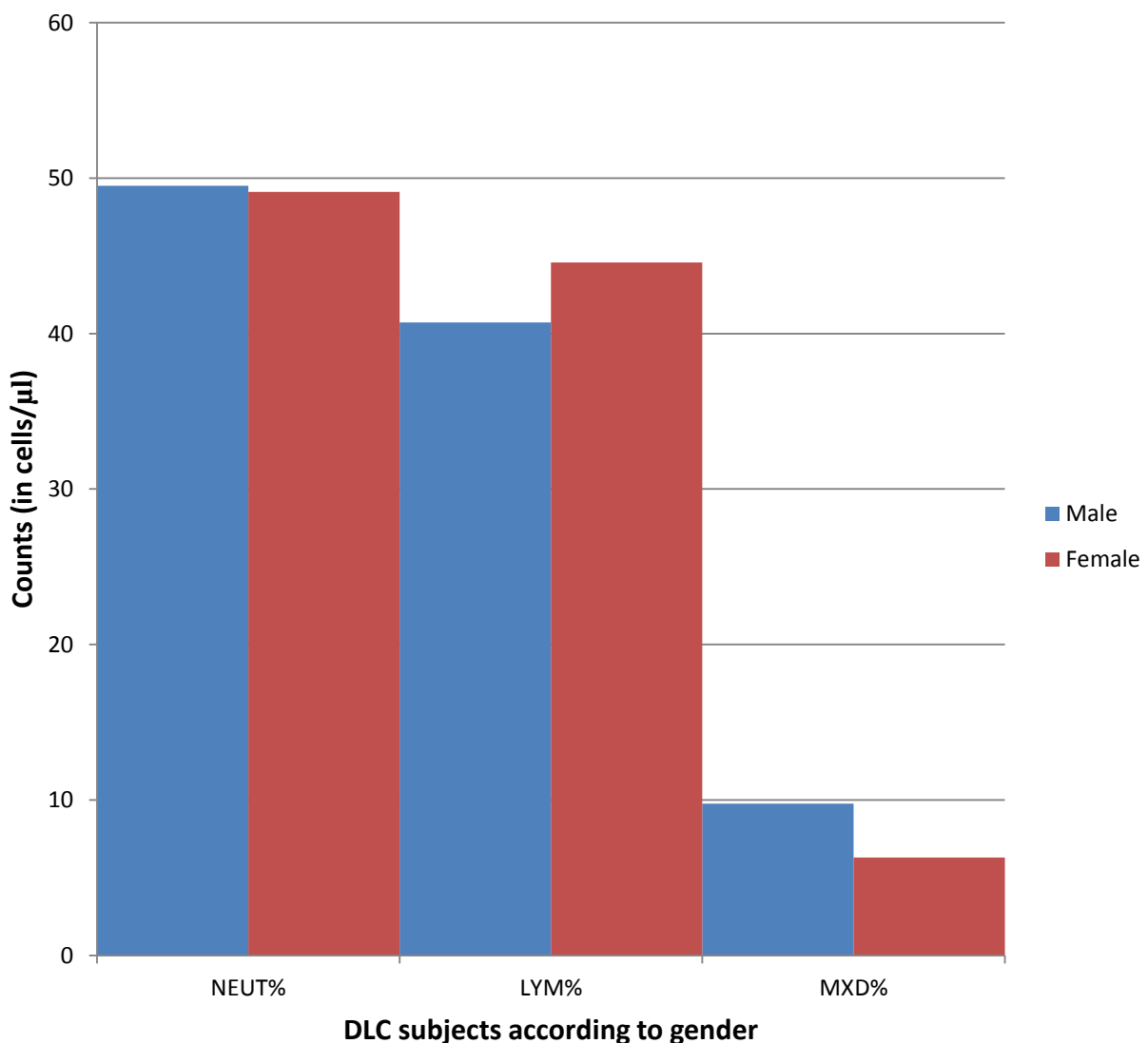


Figure 4: Differential lencocyte counts (DLC) of symptomatic subjects based on gender

KEYS:

- NEUT % - Neutrophil %
- LYM% - Lymphocyte %
- MXD% - Middle cells %

Table 5: WBC total count of HIV-1 symptomatic subjects based on age

Parameter	17-26yrs	27-36yrs	37-46yrs	47yrs & above	F-Value	P-value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
	n = 10	n = 54	n = 60	n = 20		
WBC total	3.90 ± 0.42 ^a	4.00 ± 0.55 ^a	4.23 ± 0.64 ^a	4.21 ± 0.53 ^a	0.683	0.568

KEYS:

n - Sample size

SD - Standard deviation

WBC Total - WBC count in 1µL of whole blood

Values in a row with a different superscript is significantly different at P < 0.05

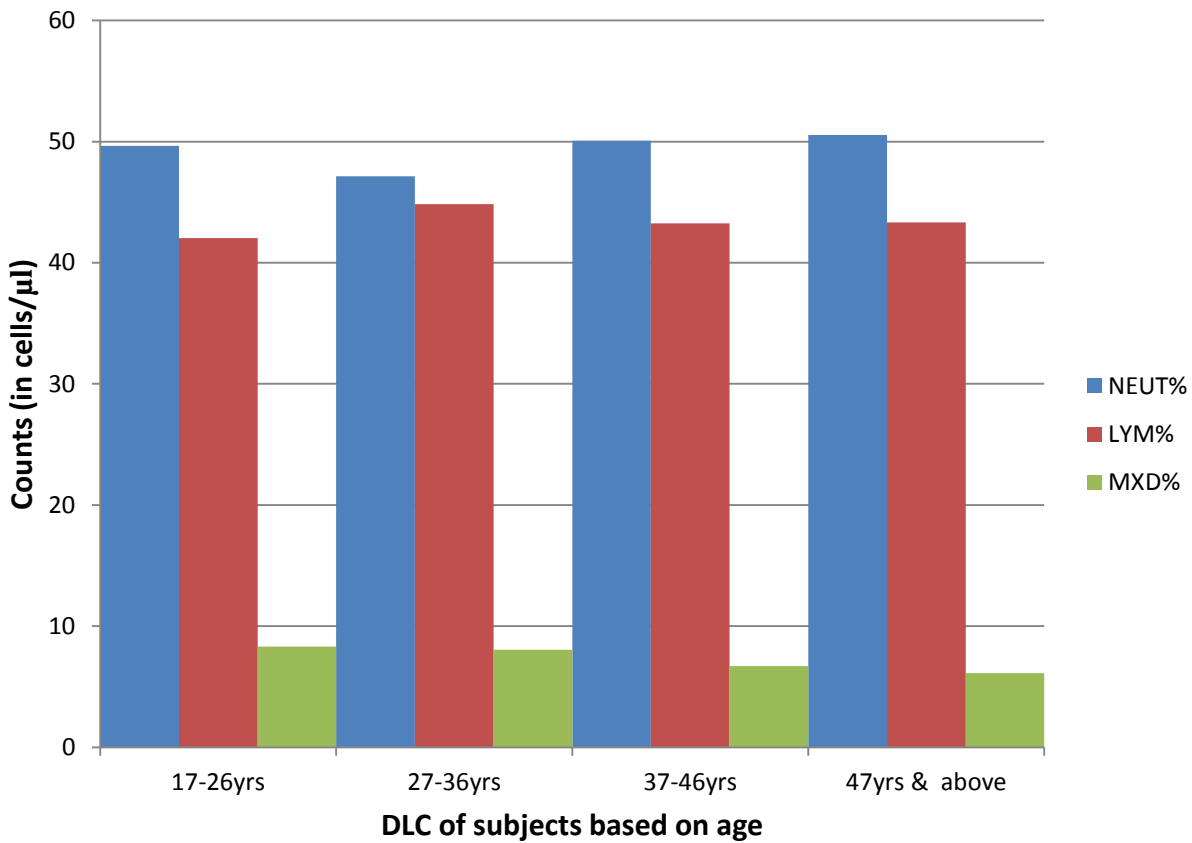


Figure 5: Histogram representation of differential counts (DLC) of symptomatic HIV – 1 subjects based on age

KEYS:

NEUT % - Neutrophil %

LYM % - Lymphocyte %

MXD % - Middle cells %

Discussion:

The number of leucocytes in the blood is often an indication of disease, and thus the WBC count is an important subset of the complete blood count. In this study, the mean WBC total count of asymptomatic HIV-1 subjects was 5.04 ± 1.14 (Table 1). Statistical comparison between the mean values of the asymptomatic HIV-1 subjects and controls revealed an insignificant difference ($P > 0.05$). This finding is in concordance with the previous report of Raman *et al.* (2016) who observed that comparison between control and HIV-infected patients did not reveal any significant difference. Our finding is also close to the observations of Dapper *et al.* (2008) who reported mean values of 4.85 ± 0.11 for healthy HIV-positive subjects in Port-Harcourt, Nigeria. It is noteworthy to mention that our results are above the lower limit of $4.0 \times 10^9/L$ of the normal reference range of total white cells and within the upper limit of the normal range of $11.0 \times 10^9/L$. On the other hand, the mean WBC total count of symptomatic HIV-1 subjects was 4.14 ± 0.56 (table 1). Statistical comparison between the mean values of the symptomatic HIV-1 subjects and control showed a statistical significant decrease ($P < 0.05$). This is in line with the reports of Obeagu *et al.* (2014) who also observed a significant decrease ($P < 0.05$) in the mean values of total white blood cells of the HIV positive patients in comparison to HIV negative subjects receiving treatment at the Federal Teaching Hospital, Abakaliki, Ebonyi State, Nigeria. Amegor *et al.* (2009) in another study on the pattern observed in WBC count (measured in cells mm^{-3}) showed that there was a significant decrease from 6.33 ± 0.33 in HIV negative subjects to 4.07 ± 0.25 in HIV positive subjects without ARVD treatments. Esan (2018) reported mean values of 4.08 ± 2.63 and 5.08 ± 1.85 in HIV positive patients (HAART and HAART naïve) revealing a statistically significant difference. Furthermore, Ifeanyichukwu *et al.* (2016) found that the WBC total ($\times 10^3/\mu L$) for HIV positive on ART was low compared to controls. Anyaechie *et al.* (2005) also reported a definite pattern of leucopenia in HIV subjects. This also supports the reports of Kumar *et al.* (2004) and Cheesbrough (2002) who both expressed that in viral infections such as HIV infection, there is a reduction in the total white blood cell count. Furthermore, Ifeanyichukwu *et al.* (2016) opined that reduced leucocyte profile might thus be an additional indication of HIV infection. Ifeanyichukwu *et al.* (2016) also

attributed the reduced levels of leucocytes among symptomatic HIV-1 subjects in these studies might also be due to generalized pancytopenia due to chronic infections like HIV. Other causes of leucopenia may also be probably due to toxicity of therapies for HIV or associated conditions. Donald and NorthFeit (1998) postulated that an autoimmune mechanism involving antigranulocytes antibodies and impaired granulopoiesis and any infiltrative process involving the bone marrow (infection and malignancy) as some of the reasons. In contrast, Raman *et al.* (2016) observed a mean value of 5.773 ± 2.270 for HIV infected cases among patients who were attending the anti-retroviral clinic in India. In another study also carried out in India, Dhal *et al.* (2018) reported a mean WBC total count of $5,872 \pm 2,210$ cells per mm^3 among HIV infected patients they studied. De Santis *et al.* (2011) reasoned that possible explanations for this finding could be that most of the clients are using HAART drugs coupled with improvements in social and nutritional conditions.

The mean neutrophil count % of asymptomatic HIV-1 subjects was 43.53 ± 8.84 (figure 4.4). Statistical comparison between the asymptomatic HIV-1 subjects and control did not reveal any significant difference ($P > 0.05$). Our finding is in line with the report of Esan *et al.* (2015) who recorded a mean neutrophil count % value of 41.53 ± 12.87 among HAART patients in Ekiti state, Nigeria. On the other hand, the mean neutrophil count % of symptomatic HIV-1 subjects was 49.25 ± 5.86 . Statistical comparison between symptomatic subjects and control revealed a significant increase ($P < 0.05$). Our finding is close to the one reported (51.52 ± 15.49) by Esan *et al.* (2015) on HAART-naïve patients in Ekiti state, Nigeria. However, other authors found a relatively higher mean neutrophil count %. For example, Dhal *et al.* (2018) recorded a mean value of 54.0 ± 12.6 in their study in India while Dapper *et al.* (2008) reported a value of 61.77 ± 0.62 for symptomatic subjects in Port-Harcourt, Nigeria. Obeagu *et al.* (2013) reported a range of 55.0 to 58.1% among HIV positive subjects across the various age groups they studied in Ebonyi State, Nigeria. These findings are also in line with another study that observed a significant increase in the median neutrophil count in comparison to baseline after HAART initiation (Mastroianni *et al.*, 1999). Researchers have showed that fundamental improvement of components of innate

antimicrobial immunity, such as neutrophils may contribute to improved cell-mediated immune responses against opportunistic infections in HAART-treated patients (Mastroianni *et al.*, 1999).

The mean lymphocyte count % of the asymptomatic HIV-1 subjects was 42.19 ± 6.25 . There was a statistical significant decrease ($P < 0.05$) in the mean value of asymptomatic HIV-1 subjects in relation to control. Our observation is in tandem with the mean value of 37.94 ± 0.75 reported by Dapper *et al.* (2008). Obeagu *et al.* (2013) also observed a range of 35.4 to 37.3% across the various age ranges of HIV subjects they studied in Abakaliki, Ebonyi State, Nigeria. However, Dhal *et al.* (2018) reported lower mean lymphocyte count (%) of $29.8 \pm 8.3\%$ among the subjects they studied in India. This may be due to suppression of the bone marrow and direct infection of T cells. This may be associated with the cytopathic effect of the immune cells in HIV positive patients.

In the present study, there was no significant difference ($P > 0.05$) in the WBC total (table 1) and differential counts of asymptomatic and symptomatic (figure 1) HIV-1 subjects according to sex in the study area. Alan and Alexander (1968) and Cruickshank (1970) reported that sex differences are insignificant in the total leucocyte count until after the age of 50 years when the count becomes less in women than men. In contrast, Dapper *et al.* (2008) observed sex differences in both the WBC total count and DLC of the subjects they studied. Bain (1996) also reported sex variations in WBC total count as well as neutrophil count. Similarly, Taylor *et al.* (1997) found slight sex differences; the total leucocyte count and the neutrophil count may be slightly higher in girls than boys. Mathur *et al.* (1979) reported that cyclic variation in WBC population during normal menstrual cycle. Apparently, menstrual cycle variations in WBC count could possibly contribute to obscuring the pattern in females likely leading to sex distinction. However, Cruickshank and Alexander (1970) and Karandikar *et al.* (2002) observed that after menopause, the counts fall in women so that they tend to become lower than in men of similar age.

With respect to age, there was no statistically significant difference ($P > 0.05$) in the mean values of WBC total and DLC of both asymptomatic (table 3 and figure 3) and symptomatic (table 5 and figure 5) HIV-1 subjects. Our finding is in line with

the reports of Obeagu *et al.* (2014) who found no significant difference ($P > 0.05$) in the differential count (DLC) of the subjects they studied in Abakaliki, Ebonyi State, Nigeria. However, Taylor *et al.* (1997) observed that from the 5th to 7th years when lymphocytes give way to the neutrophils, the levels are the same as for adults inferring that age does not necessarily affect the WBC total and differential counts of adults.

In conclusion, the WBC total count was not significantly reduced ($P > 0.05$) among asymptomatic HIV-1 subjects whereas it was significantly reduced ($P < 0.05$) among symptomatic HIV-1 subjects. Age and sex did not affect the WBC total count of both subjects in the study. In addition, the neutrophil count % was not increased ($P > 0.05$) among asymptomatic HIV-1 subjects. In contrast, there was a significant increase ($P < 0.05$) in the neutrophil count % in relation to lymphocyte count % of symptomatic HIV-1 subjects. Furthermore, there was no significant difference ($P > 0.05$) in the lymphocyte count % of both subjects. Age and sex differences did not also occur in the DLC of both subjects. This study has further confirmed the prognostic role of leucocyte counts (WBC total and neutrophil counts %) can play especially in low-resource areas in the diagnosis of symptomatic HIV-1 subjects in the study area.

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