

NEUTROPHIL-LYMPHOCYTE AND PLATELET-LYMPHOCYTE RATIOS OF PREGNANT WOMEN LIVING WITH HIV/AIDS.

ABSTRACT

Background: Chronic inflammation, a factor for high mortality, persists throughout the pregnancy period of women living with human immunodeficiency Virus (HIV).

Aim: To determine whether pregnancy alone or the combined effects of HIV and pregnancy are the sources of the inflammation, this study evaluated the predictive variables for inflammation, lymphocyte ratio (NLR), and Platelet-Lymphocyte Ratio (PLR).

Material and method: A population of 128 women, aged 15-49 years were grouped into 4; Group A- 32 HIV positive and pregnant, Group B- 32 HIV negative and pregnant, Group C- 32 HIV positive not pregnant, Group D- 32 HIV negative not pregnant (control group). Differential white blood cell and platelet count methods were used to determine the neutrophil (N), Lymphocyte (L) and platelet (P) counts. The ratio calculation method was used to derive the NLR and PLR.

Results: NLR was significantly higher for group A (2.62 ± 0.41) compared to groups B (2.25 ± 0.18); C (0.95 ± 0.07); D (1.68 ± 0.19) at $p \leq 0.00$. However, the NLR of groups A and B ($p \leq 0.05$) were not significantly different, PLR was significantly higher for group A (9.86 ± 1.12) compared to groups B (6.65 ± 1.12); C (5.35 ± 0.47); D (6.34 ± 0.43). There was no significant difference in blood pressure across the groups, significant difference was however observed in their Body Mass Index.

Conclusion: Hence, Inflammation may have been caused by pregnancy alone.

Keywords: Inflammation, Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio, Human immunodeficiency virus, pregnancy.

Introduction

The human immunodeficiency virus (HIV) epidemic continues to affect young women within the reproductive age with a prevalence of 4.1% among pregnant women in Nigeria, while HIV prevalence is 1.4% for the age range; 15-49 years (1). Since 2010, pregnancy has become an indication for the use of triple antiretroviral (ARV) drugs for prophylaxis or therapy in HIV-positive women in Nigeria (2). Many studies were carried out to assess the effect of these (HIV and ARV drugs) on the hematological parameters. However, they were mainly done in isolation, and there is a paucity of data on the combined effect of HIV, and pregnancy on the haematological parameters (neutrophil-lymphocyte and platelet-lymphocyte ratio), especially in this part of the country.

Neutrophils (also known as neutrocytes or heterophils) are the most abundant type of granulocytes and make up 40% to 70% of all white blood cells in humans (3). They are one of the first responders of inflammatory cells to migrate toward the site of inflammation (4).

A Lymphocyte is a type of white blood cell in the immune system of jawed vertebrates (5). They include natural killer cells (which function in cell-mediated, cytotoxic innate immunity), T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity) (6).

Platelets, also known as thrombocytes are a component of blood whose function is to react to bleeding from blood vessel injury by clumping. Thereby initiating a blood clot (7). Platelets are a major source of TGF- β 1 and release this cytokine upon activation and degranulation, along with alpha-granule chemokines such as CXCL4, also known as platelet factor 4 (PF4) (8). Platelets have a significant role in modulating clot formation. Additionally, emerging data indicates that platelets have considerable roles in inflammation and immune response. Platelets gather at the damaged site and adhere to white blood cells. Subsequently, they release cytokines and chemokines which are chemotactic for neutrophils and monocytes. Therefore, platelets

are necessary for targeting lymphocytes, neutrophils, and monocytes at the inflammation site. Those interactions enhance inflammation (9). The Neutrophil/Lymphocyte ratio, and Platelet/Lymphocyte ratio are therefore considered to be prognostic factors for inflammation (chronic and acute) and immunity (adaptive), which are major health concerns for HIV patients.

A complete blood count (CBC) test is a simple economical and extensively used basic hematological test which mainly included white blood cell (WBC) count, red blood cell (RBC) count, and platelet count (10). The most abundant white blood cells in healthy humans are neutrophils, which play important roles during acute and chronic inflammation and may be potential therapeutic targets in cardiovascular diseases (11). The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are the proportions of absolute neutrophil-to-lymphocyte and platelet-to-lymphocyte counts retrieved from the CBC test. As markers of inflammation, various studies have demonstrated the correlation between NLR, PLR, and many diseases such as inflammatory diseases (12) cardiovascular diseases, cancer, and long-term type 2 diabetes remission after metabolic surgery (13).

This study aimed to evaluate the Neutrophil-Lymphocyte ratios and platelet-lymphocyte ratios (prognostic factors for inflammation) of pregnant women living with HIV and compare with other groups of women to determine if pregnancy alone was the cause of inflammation or the combined effect of pregnancy and HIV.

Materials and methods

Sample collection:

5ml of venous blood was obtained from the ante cubital fossa of each participant using a sterile needle and syringe. They were then dispensed into appropriate sample bottles of ethylene diamine tetra acetic acid (EDTA) for Neutrophil, Lymphocyte and Platelet count determination. Samples were stored at 20°C until analysis

Equipment: Oil immersion Microscope (Wincom - China), Weighing balance (Boldfit – USA), Sphygmomanometer (Accoson – UK).

Reagent: Hemolytic agents, diluents, cleaning solutions and concentrated cleaning solutions (Dirui, China), Distilled Water, Immersion Oil (Wincom – China).

Study Area:

This study was carried out at the antenatal care (ANC) unit of Nnamdi Azikiwe University Teaching Hospital, Nnewi in Nnewi North local government area, about 25km south of Onitsha in Anambra State, between latitudes 5⁰59' 41.64"N and 6⁰03' 28.44"N and longitudes 6⁰03' 28.44"E and 6⁰52' 41.64"E, bounded in the north by Idemmili South, west by Ekwusigo and South East by Nnewi South L.G.As of Anambra State.

Study Population

The participants were 128 women. Comprising of 64 women living with HIV/AIDS, where 32 were pregnant women and 32 not pregnant. Another 64 were the women not living with HIV/AIDS, where 32 were pregnant women and 32 not pregnant. All in Nnewi North Local Government Area and Dunukofia local government area, both in Anambra State. This population are clustered at antenatal care (ANC) unit of Nnamdi Azikiwe University Teaching Hospital, Nnewi and Ukp.

Population Grouping

- Group A HIV positive, Pregnant Women (study group)
- Group B HIV negative, Pregnant Women
- Group C HIV positive, non-Pregnant Women
- Group D HIV Negative, non-Pregnant Women (control group)

Inclusion Criteria

- Pregnant women living with HIV/AIDS.
- Pregnant women but not living without HIV.
- Women living with HIV but not pregnant
- Women not living with HIV and also not pregnant.
- Age range: 15-49 years.

Exclusion Criteria

- The pregnant women that were excluded from the study:
- Those living with HIV/AIDS but are not on antiretroviral drugs

Sample Size

Sample size was calculated using G*Power software version 3.0.10. (Universitat Dusseldorf Germany). Power analysis for one-way Analysis of Variance (ANOVA) with 2 groups was carried out to determine a sufficient sample size using an alpha error probability of 0.05, a power of 90 and a medium effect size. Based on these, the calculated total sample size was a total of 128 participants (minimum sample size that would be required for a reasonable statistical inference to be made from the study) comprised of 64 HIV positive women and 64 HIV negative women. Within, the groups were further divided into two. The HIV negative women into 32 pregnant and non-pregnant, then the HIV positive women into 32 pregnant and 32 non-pregnant. The study group are the pregnant women living with HIV.

Anthropometric Indices

Heights were taking using measuring tape (in meters), weights taking using weighing balance (in kg), Body mass index (BMI) calculated as Kg/m^2 and Blood pressure (BP) were taking using Sphygmomanometer (systolic blood pressure/diastolic blood pressure)

Blood Testing

The Differential White Blood Cell and Platelet Counts(14)

For Platelet count, repeat same procedure as in Neutrophil and Lymphocyte counts. But ignore all white blood cells entirely and count only the platelets.

Neutrophil and Lymphocyte Counts:

The relative percentage of each type of white blood cell was determined after a total of 100 white blood cells have been identified. The Neutrophils are the most abundant, followed by the lymphocytes.

Prepare slide,

Perform Cell Count; Oil immersion lens of the microscope was used (high-dry optics was not used because it makes the differentiation of some cells difficult).

Calculation of Neutrophil-Lymphocyte and Platelet-Lymphocyte ratio(15)

Calculation of Neutrophil-Lymphocyte Ratio

$$\text{NLR} = \frac{\text{Absolute Neutrophils}}{\text{Absolute Lymphocytes}} = \frac{\text{Relative \% Neutrophils}}{\text{Relative \% Lymphocytes}}$$

Calculation of Platelet-Lymphocyte ratio:

$$\text{PLR} = \frac{\text{Absolute Platelet}}{\text{Absolute Lymphocytes}} = \frac{\text{Relative \% Platelet}}{\text{Relative \% Lymphocytes}}$$

Statistical Analysis: Data analysis was conducted out using Statistical Package for the Social Sciences (SPSS) version 23 (IBM Corp., Amonk, NY). Results was expressed as mean and standard error of mean ($\bar{X} \pm \text{SEM}$). One-Way Analysis of Variance (ANOVA) was used to compare between means. P-value of ≤ 0.05 was considered.

Results

Table 1, presents the result of the Neutrophil, Lymphocyte and Platelet counts for the Groups A, B, C and D. For all the groups, the values for the parameters were within the normal range (16). There was a significant difference in values of these parameters across the groups. For Neutrophil, Group B recorded highest value, followed by Group A, then Group D and C. For Lymphocyte, Group C recorded highest value, followed by Group D, then Group A and B. For Platelet, Group A recorded highest value, followed Group C, then Group D and B.

Table 2, presents the result of the Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio, Systolic and Diastolic Blood pressure and Body mass index. Across the groups, there was a significant difference in the NLR, PLR and BMI but not in their Systolic and Diastolic blood pressure. For NLR, Group A had highest value, followed by Group B, D and C. For PLR, Group A had the highest value, followed by Group B, D and C. For the BMI, Group B was the highest, followed by Group C, D and A.

Table 3, presents the result of multiple Comparisons of Neutrophil, Lymphocyte, Platelet, Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio, BMI, Systolic and Diastolic Blood Pressures for Groups A, B, C and D.

Group A compared to Group B, there is a significant mean difference for their Platelet, Platelet-Lymphocyte ratio and BMI.

Group A compared to Group C, there is a significant mean difference for their Neutrophil, Lymphocyte, Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio and BMI.

Group A compared to Group D, there is a significant mean difference for their Neutrophil, Platelet, Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio and BMI.

Group B compared to Group C, there is a significant mean difference for their Lymphocyte and Platelet.

Group B compared to Group D, there is only a significant mean difference for their Neutrophil.

Group C compared to Group D, there is a significant mean difference for their Neutrophil, Lymphocyte and Neutrophil-Lymphocyte ratio.

Table 1: Neutrophil, Lymphocyte and Platelet counts for all groups

	Group A	Group B	Group C	Group D	F-value	p-value
N (%)	58.02±2.22	59.58±1.73	39.98±1.60	48.88±1.39	26.625	0.000*
L (%)	29.91±1.92	29.68±1.43	44.93±1.31	33.73±1.57	20.619	0.000*
P (x10⁹/L)	239.53±8.49	179.75±8.24	226.53±11.81	195.47±5.55	9.756	0.000*

Values are presented as Mean ± SEM, *p-value* ≤ 0.05. *(significant)

Keys: N.- Neutrophil, L – Lymphocyte, P – Platelet, Group A- HIV positive, pregnant women, Group B- HIV negative, pregnant women, Group C- HIV positive, non-pregnant women, Group D- HIV negative, non-pregnant women.

Table 2: The Neutrophil-Lymphocyte Ratio, Platelet-Lymphocyte Ratio, Systolic Blood Pressure, Diastolic Blood Pressure and BMI index of all the groups.

	Group A	Group B	Group C	Group D	F-value	P-value
NLR	2.62±0.41	2.25±0.18	0.95±0.07	1.68±0.19	8.907	0.000*

PLR (x10 ⁷ L)	9.86±1.12	6.65±0.55	5.35±0.47	6.34±0.43	7.783	0.000*
SBP (mmHg)	126.75±4.72	125.72±4.70	132.63±4.57	126.66±3.25	0.527	0.665 ^a
DBP (mmHg)	73.41±1.43	75.59±1.69	76.09±1.86	75.47±1.78	0.491	0.689 ^a
BMI (Kg/m ²)	25.69±0.43	29.46±0.69	28.17±0.58	28.09±0.61	7.212	0.000*

Values are presented as Mean ± SEM, *p-value* ≤ 0.05. *(significant), ^a(not significant)

Keys: NLR – Neutrophil-Lymphocyte Ratio, PLR – Platelet-Lymphocyte Ratio, SBP – Systolic Blood Pressure, DBP – Diastolic Blood Pressure, BMI – Body Mass Index. Group A- HIV positive, pregnant women, Group B- HIV negative, pregnant women, Group C- HIV positive, non-pregnant women, Group D- HIV negative, non-pregnant women.

Table 3: Multiple Comparisons of Neutrophil, Lymphocyte, Platelet, Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio, BMI, Systolic and Diastolic Blood Pressures for Groups A, B, C and D

	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
N %	0.530 ^a	0.000*	0.000*	0.530 ^a	0.000*	0.000*

L %	0.918 ^a	0.000*	0.089 ^a	0.000*	0.072 ^a	0.000*
P x10⁹L	0.000*	0.296 ^a	0.001*	0.000*	0.209 ^a	0.014*
NLR	0.286 ^a	0.000*	0.007*	0.000*	0.098 ^a	0.038*
PLR x10⁹ L	0.002*	0.000*	0.001*	0.194 ^a	0.754 ^a	0.322 ^a
SBP mmHg	0.867 ^a	0.342 ^a	0.988 ^a	0.264 ^a	0.879 ^a	0.334 ^a
DBP mmHg	0.363 ^a	0.264 ^a	0.391 ^a	0.835 ^a	0.958 ^a	0.795 ^a
BMI Kg/m²	0.000*	0.003*	0.004*	0.121 ^a	0.103 ^a	0.932 ^a

p-value \leq 0.05, ^anot significant, *Significant

Keys: N- Neutrophil, L- Lymphocyte, P- Platelet, NLR – Neutrophil-Lymphocyte Ratio, PLR – Platelet-Lymphocyte Ratio, SBP – Systolic Blood Pressure, DBP– Diastolic Blood Pressure, BMI – Body Mass Index. Group A- HIV positive, pregnant women, Group B- HIV negative, pregnant women, Group C- HIV positive, non-pregnant women, Group D- HIV negative, non-pregnant women.

Discussion

This study analyzed the mean values of Neutrophil, Lymphocyte, Platelet, Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio, Systolic/Diastolic blood pressure, and Body mass index of the study group, further, compared the values with other groups and the control.

In this study, the Neutrophil, Lymphocyte, and Platelet Counts of all the groups were within the normal range. The normal range for Neutrophil, lymphocyte, and Platelet counts are 40-75%, 20-45%, and 100-300 x 10⁹/L respectively (16). However, there is a significant difference in the Neutrophil, Lymphocyte, and Platelet counts of all the groups. Pregnant women (Group A and B), both HIV positive and Negative had higher Neutrophil count, followed by Group D, women not living with HIV and not pregnant, then Group C, women living with HIV but not pregnant. This suggests that the rise may be because of pregnancy and not due to HIV condition. This finding of a significant increase in Neutrophil value in HIV pregnant women as compared to other groups does not agree with some other findings (17,18), theirs showed that pregnancy in HIV patients is associated with low leukocytes. For the Lymphocyte count, Pregnant women (Group A and B), both HIV positive and Negative had low lymphocyte count, followed by Group D, women not living with HIV and not pregnant, then Group C, women living with HIV but not pregnant. Note that a lower lymphocyte value will invariably result in an elevated Neutrophil-Lymphocyte ratio (19). There is, however, a different observation on the platelet counts. The values for Group A and Group C (the women living with HIV) had higher platelet counts compared to Group B and Group D (the women not living with HIV). The underlying factor is not known since several studies (20, 21,22) suggest that HIV is associated with Low

platelet count and also proves clearly that platelet count can only be high in HIV patients whose disease state had progressed and are not on antiretroviral drugs.

There was a significant difference in Neutrophil-Lymphocyte Ratio in all the groups. The neutrophil-lymphocyte ratio was highest in pregnant women living with HIV, followed by pregnant women not living with HIV, then women not living with HIV and not pregnant, and lastly women living with HIV but not pregnant. This points to pregnancy but not HIV or antiretroviral drugs as contributing to the significant increase in the Neutrophil-Lymphocyte ratio. This agrees with an earlier work (23), which reported that pregnancy alone can cause a decrease in lymphocyte level, which invariably results in a high Neutrophil-Lymphocyte ratio. Though in this study, the Neutrophil-Lymphocyte ratio was observed to be highest in pregnant women living with HIV, it is not considered to be elevated, NLR is considered elevated at ≥ 6 (24). A normal range of NLR is between 1-2, values higher than 3.0 and below 0.7 in adults are pathological. NLR in a grey zone between 2.3-3.0 may serve as an early warning of pathological states or processes such as cancer, atherosclerosis, infection, inflammation, psychiatric disorders, and stress (24). Also, the normal NLR values in an adult, non-geriatric, population in good health are between 0.78 and 3.53 (25). Therefore, from the findings in this work, the mean value of the Neutrophil-Lymphocyte ratio at 2.62 ± 0.41 for the study group is an indication of inflammation and mild stress. But for the control group, the neutrophil-lymphocyte ratio at 1.68 ± 0.19 is within the normal range, below the grey zone.

The platelet-lymphocyte ratio was highest in pregnant women living with HIV, followed by pregnant women not living with HIV, then women not living with HIV and not pregnant, and lastly women living with HIV but not pregnant. This suggests that pregnancy (but not HIV or antiretroviral drug) and elevated blood pressure contributed to the significant increase in the Platelet-Lymphocyte ratio, hence, the positive correlation between the Platelet-Lymphocyte ratio and the systolic/diastolic blood pressure was observed. A blood pressure of $\geq 130/80$ is considered high, this is called hypertension, while a blood pressure between 120 and 129 and less than 80 is considered elevated (26). Which suggests the risk of developing hypertension. The inference that the increase in the Platelet-Lymphocyte ratio of pregnant women living with HIV could be due to pregnancy and elevated blood pressure agrees with a report that the Platelet-Lymphocyte ratio was higher in women with preeclampsia (high blood pressure in pregnancy) condition (27).

The BMI across the groups was significantly different. Group B recorded the highest value, followed by Group C, then by Group D, and lastly Group A. Generally, for all groups, their BMI is considered to be overweight (28). Though overweight or obesity had been associated with inflammation (29), it cannot be considered as an underlying cause for inflammation here, since the study group that suffers the inflammation had the lowest BMI compared to other groups, yet their cells are not inflamed.

It was also observed that the blood pressure (systolic/diastolic blood pressure) of the participants across the groups were all elevated (30) and not significantly different at p-value 0.665 for systolic blood pressure and 0.689 for the diastolic blood pressure. This eliminates blood pressure as a factor that may affect the Neutrophil, Lymphocyte, and Platelet counts of the groups.

Conclusion

The results from this study showed all comparisons between the study group (Group A); HIV pregnant women and Group B; HIV negative, pregnant women to be similar and for most factors not significantly different, therefore, suggests that the persistent inflammation found in pregnant women living with HIV was caused by pregnancy alone.

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