JOURNAL OF THE MEDICAL WOMEN'S ASSOCIATION OF NIGERIA

Established in 2004 March 2025 Volume 10: No 1

Original Article

Access this article online

Quick Response Code:

Website: www.jmwan.org.ng

DOI: https://doi.org/10.71526/jmwan.v10i1.66

Comparative Assessment of Neutrophil Counts and use of Neutropenia to predict Low CD4 Count in Anti-Retroviral Therapy (Art)-Naive HIV positive Children in Enugu, Nigeria

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Abstract

BACKGROUND: Neutropenia has been noted to be associated with HIV disease progression and reduced survival in children. Neutropenia has been postulated to be associated with low CD4 count in HIV-positive children by some researchers. Hence, this study sought to assess the prevalence of neutropenia in ART-naïve HIV-positive patients and compare them to HIV-negative subjects and also to investigate if reduced absolute neutrophil counts can be used to predict low CD4 count in the HIV-positive patients.

METHODS: This study was a prospective observational study carried out at the University of Nigeria Teaching Hospital, Enugu, involving a total of 200 children (100 test subjects and 100 test controls). Biodata was collected with a proforma and blood samples were subsequently collected and analysed for neutrophil count and CD_4 count. Data generated were analysed with Statistical Package for Social Sciences (SPSS) version 22.

RESULTS: The result showed that neutropenia with a prevalence of 18% is not significantly associated with HIV infections. It was also shown that there is no significant association between the age of the children and neutropenia. Likewise, CD4 count was not significantly associated with neutropenia.

CONCLUSION: The study showed that neutropenia in the subjects is comparable with that of the controls and cannot be used as a substitute for CD_4 count or as one of the criteria for commencement of antiretroviral medications in ART-naive HIV-positive children.

Keywords:

HIV, antiretroviral therapy naïve, neutropenia, CD_4 count, children.

Introduction

The Human Immunodeficiency Virus (HIV) is one of the most important emerging infections of this century.[1] The HIV infection causes the Acquired Immunodeficiency Syndrome (AIDS), which is a systemic disorder characterised by deficiency in cellular immune responses.[1] It is probably one of the diseases with multiple impacts on persons, families, communities and the entire society.[1] HIV is threatening, especially in sub-Saharan African countries. In Nigeria, the prevalence rose from 3.8% in 2003 to 5.8% in 2011.[1] Haematological complications such as neutropenia have been documented to be the second most common cause of morbidity and mortality in HIV-positive persons.[2] A variety of haematological manifestations are seen at every stage of HIV and often life-threatening and impair the quality of life of these patients.[3]

Haematological abnormalities such as neutropenia have been documented as strong independent predictors of morbidity and mortality in HIV-infected children.[3] Although it is not part of the criteria for initiating therapy nor used by the World Health Organisation (WHO) for staging HIV, peripheral blood cell abnormalities in an abnormal haemogram are important prognostic tools for morbidity in HIV infection and AIDS.[3] The 2011 Mbanya *et al*.[4] study at the Central Hospital of Yaoundé, done among ART-naïve AIDS patients, revealed that 95.4% of AIDS patients had morphological blood cell disorders compared to 32.2% amongst healthy controls. Haematological manifestations in HIV-infected patients often pose a great challenge in the comprehensive management of such patients and may cause symptoms that are life-threatening and impair the quality of life of these patients.[5]

The haematological complications, such as neutropenia, are associated with HIV disease progression and subsequent reduced survival.[5] It has been noted that a large decline in neutrophil count in HIV-positive patients is a pointer for later development of HIV-induced central nervous system (CNS) disease, which is by all means catastrophic to the patients.[6] This complication in HIV-infected ART-naïve patients usually results in poor ART treatment outcomes when they eventually become eligible for ART and strongly predicts poor prognosis and increased mortality.[6] This complication, when present, must be addressed appropriately and in good time.

Neutropenia is defined as a neutrophil count less than 1500 cells/mm^3 and is the most common leucopenia occurring in HIV-infected individuals.[7] Neutropenia is common and its incidence rises from 13% to 44% with disease progression from HIV to AIDS.[7] HIV infection suppresses the bone marrow and leads to decreased levels of granulocyte colony-stimulating factor, the factor that stimulates production of white blood cells in the bone marrow and affects the granulocyte-macrophage lineage, resulting in leucopenia and neutropenia.[8] Neutropenia can also result from other opportunistic infections, including cytomegalovirus, tuberculosis, histoplasmosis and leishmaniasis.[15] Hence, HIV patients with cytopenias may require bone marrow examination to determine the cause and to direct therapy.[8]

Low CD_4 count is defined as a CD4 count less than 500 cells/mm^3. Low CD_4 count has been associated with prevalent anaemia, neutropenia and thrombocytopenia.[9] HIV preferentially infects CD_4 cells, causing their destruction.[10] It has been calculated that each day, more than 1 billion CD_4 cells are destroyed.[10] The decline in CD_4 count is linked to viral load and is used as a measure of disease progression.[10]

Neutropenia has been associated with a low baseline CD_4 count.[11] Taking into account the patient's CD_4 cell count, Suresh *et al*.[11] showed that the incidence of neutropenia was 0.8% in HIV patients with CD_4 count greater than 700 cells/mm^3 and rose to 13.4% in those with CD_4 count less than 250 cells/mm^3.

Hence, it is concluded that there is a strong negative association between CD_4 counts and the severity of neutropenia, anaemia and thrombocytopenia in HIV patients.[12] Therefore, a complete blood count with differential should be performed every three months to screen for haematological abnormalities in HIV-infected children. A complete blood count may need to be obtained more often for children receiving bone

Print ISSN: 3043-4742 Online ISSN: 3043-4750

marrow suppressive therapy or if abnormalities are identified.

Although neutropenia is a common manifestation of HIV infection and may have considerable impact on patients' well-being, treatment and care, few studies on neutrophil counts in HIV-infected children have been undertaken in Africa, but none in Nigeria in particular and Enugu in general, to the best of the author's knowledge. Such information for HIV-infected children in Enugu may help to forestall potential treatment failure later.

By way of justification, Africa is the epicentre of the HIV epidemic, as reported by the World Health Organisation (WHO), and Sub-Saharan Africa contributes about 67% of the burden worldwide.[1] About 22 million people were living with the virus by the end of 2007.[1] Nigeria has the largest number of people living with HIV/AIDS in West Africa and the second largest in the subcontinent.[1] Neutropenia is associated with HIV disease progression and subsequent reduced survival.[2] It has been noted that a large decline in neutrophil counts in HIV-positive patients is a pointer for later development of HIV-induced CNS disease, which is by all means catastrophic to the patients.[6] This complication in HIV-infected ART-naïve patients usually results in poor ART treatment outcomes when they eventually become eligible for ART and strongly predicts poor prognosis and increased mortality. This complication, when present, must be addressed appropriately and in good time. Studies on neutrophil counts in HIV-infected children are almost non-existent in Nigeria, hence the compelling need to carry out this vital and essential study and hopefully go a long way in averting the potential problems this might cause to the patients. This study will also provide baseline information for the country as well as provide data on which further studies on the topic can be developed.

The aim of this research is to study the effects of human immunodeficiency virus (HIV) infection on neutrophil count in HIV-positive ART-naive children seen at University of Nigeria Teaching Hospital (UNTH), Enugu, while the objectives are as follows: to assess the level of neutrophil counts in ART-naive HIV-positive patients and compare it to the uninfected patients (control); to determine the prevalence of neutropenia in ART-naive HIV-positive patients; and to relate the level of neutrophil counts with the level of CD4 counts in ART-naïve HIV-positive patients.

Materials and Methods

Study Design: This was a prospective observational study conducted at a tertiary health facility with paediatric HIV/AIDS facilities: the University of Nigeria Teaching Hospital, Enugu. Paediatric HIV/AIDS clinics usually run once a week. Subjects were enrolled as they presented to the clinic for the first time or for a check-up. Information about biodata, medical data and demography were obtained from all the children under study through their parents or guardians using a proforma designed by the researcher. Subjects and controls were physically examined and their axillary temperature checked with a mercury thermometer to exclude those with febrile conditions. Their weights were also measured in kilograms and their weight-for-age centiles were used to determine their nutritional status and hence exclude those that were malnourished using modified welcome classification. Neutrophil counts were determined on subjects and controls.

Study Population:

Subjects: All ART-naïve HIV positive children

Controls: All HIV negative children

Sample Size Estimation

Sample selection was by consecutive enrolment.

Sample size was calculated using the formula[15]:

$$n = \frac{Z^2 pq}{d^2}.$$

Where n = Sample Size

Z = 1.96 for 95% Confidence interval

P = Proportion with the characteristic (HIV positive subjects) = 5.8% (WHO, 2012).

q = Proportion without the characteristic = 1 – 0.058

= 0.942

d = Degree of accuracy, usually set at 0.05.

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$$n = \frac{1.96^2 \times 0.058 \times 0.942}{0.05^2}$$

n = 83.96

Attrition rate = 10% of calculated sample size

Minimal sample size = 92.36

However, sample size of 100 was used.

Inclusion Criteria:

1. All HIV-positive ART-naïve children who were attending the Paediatric HIV/AIDS clinic at UNTH, Enugu.

2. All HIV-positive ART-naïve children of consenting mothers.

Exclusion Criteria:

1. HIV-positive children who have commenced ART.

2. HIV-positive ART-naïve children with inconsistent neutrophil counts.

3. Malnourished or febrile HIV-positive ART-naïve children.

4. HIV positive ART-naïve children on medications that have the potential to cause myelosuppression such as anti-cancer drugs.

Data Presentation and Analysis: Data generated were entered into the proforma by the researcher and were subsequently entered into a computer data base. The data were then analyzed with Statistical Package for Social Sciences (SPSS) version 22 of the IBM Corporation, 1 New Orchard Rd, Armonk, NY 10504.

Descriptive statistics such as frequency, percentages, mean, median and standard deviations were used to summarise categorical and continuous variables. Median values of neutrophil count (skewed variable) were compared between subjects and age/sex-matched controls using the Mann-Whitney U Test. Relationships between age, CD4 count and neutrophil counts were done using correlation and linear regression. Associations between HIV status, different levels of CD4 count and neutrophil counts were tested using logistic regression and chi-square. The statistical significance was set at $p < 0.05$. Results were presented in tables and figures.

Laboratory Methods: After obtaining an informed consent from the mothers/guardians, blood samples (about 2 mL each) were collected from peripheral veins into an EDTA-containing sample bottle and rocked severally to allow for proper mixture of blood and anticoagulant. The samples were then analysed by the researcher using the autoanalyser under the supervision of a laboratory scientist. Another blood sample, 2 mL each, were collected two weeks after from the same subjects, and subjects with consistent values on both occasions were enrolled.

Ethical Consideration: The approval of the Health Research Ethics Committee of the University of Nigeria Teaching Hospital, Enugu was obtained. Informed consent to participate was obtained from all of the participants during investigations.

Results

Table 1: Sex and age distribution of subjects and controls

	Subjects		Control	
Age (group)	**Male**	**Female**	**Male**	**Female**
1 – 3	19 (39.6)	14 (26.9)	19 (39.6)	14 (26.9)
4 – 6	9 (18.8)	15 (28.8)	9 (18.8)	15 (28.8)
7 – 9	14 (29.2)	16 (30.8)	14 (29.2)	16 (30.8)
10 – 12	6 (12.5)	7 (13.5)	6 (12.5)	7 (13.5)
Total	**48 (100)**	**52 (100)**	**48 (100)**	**52 (100)**

$X^2 = 2.332$, $p = 0.431$

A total of 200 children (100 subjects and 100 controls) were enrolled into the study. All matched for sex and age. There were 48 males and 52 females with ratio of approximately 1:1 but the gender difference was not statistically significant. ($p = 0.431$). The ages of the children

Print ISSN: 3043-4742 Online ISSN: 3043-4750

were grouped as shown in Table l. The age ranged from 1 to 12 years with a mean ± standard deviation (SD) of 5.59 ± 3.25 years. The predominant ages were in the 1-3 years group while the least predominant ages were in the 10-12 years group. (Table 1)

Table 2: Comparison of absolute neutrophil count in the Subjects and Controls

Parameters	**Subjects** Median (Mean rank)	**Controls** Median (Mean rank)	Mann-Whitney U	p-value
Neutrophil counts	2428.50 (90.51)	3320.50 (110.50)	4000.50	0.015

In the subjects, the Neutrophil Counts ranged from 572 to 18711 cells/mm3 with a mean ± standard deviation (SD) of 3176 ± 2469 cells/mm^3 .In the controls, the Neutrophil Counts ranged from 105 to 12450 cells/mm^3 with a mean ± standard deviation of 3966.14 ± 2646.14 cells/mm^3.

The neutrophil counts were not normally distributed in both the subjects and the controls. Therefore, a non-parametric alternative of t-test which is the Mann-Whitney U test was used to determine the association of ANC between the subjects and controls.

Median neutrophil count for subjects (2428.50) when compared to that of controls (3320.00) were significantly different. (p = 0.015). (Table 2)

Table 3: Prevalence of neutropenia and its association with Subjects and Controls.

	Subject	**Control**	**p value**	**OR**	**95% C.I for OR**
Neutrophil Counts					
Neutropenia	18	14	0.441	1.348	0.630 – 2.887
Normal	82	86			

Neutropenia was seen in 18 subjects (11 males and 7 females) and in 14 controls (9 males and 5 females). Prevalence of neutropenia is 18% among the subjects and 14% among the controls. There is no significant association in the prevalence of neutropenia between the subjects and the controls (p = 0.441). (Table 3)

Table 4: Comparison of absolute neutrophil count in Male and Female Subjects and Controls

Parameters	**Gender** **Male** Median (Mean rank)	**Female** Median (Mean rank)	Mann-Whitney U	p-value
Neutrophil count (Subjects)	2394.00 (48.05)	2472.50 (52.76)	1130.50	0.418
Neutrophil count (Controls)	3138.00 (46.29)	(3407.00 (54.08)	1048.50	0.181

In the subjects, there is no significant difference between: Gender and Absolute Neutrophil Count, p = 0.418. In the controls also, there is no significant difference between: Gender and Absolute Neutrophil Count, p = 0.181. (Table 4)

Table 5: Relationship between age and neutrophil counts in subjects and controls

	Statistics	Neutrophil counts (cell/mm^3) **Subjects**	Neutrophil counts (cell/mm^3) **Controls**
Age	Pearson Correlation (r)	-0.110	0.046
	p value	0.277	0.651
	N	100	100

There is no significant relationship between the neutrophil counts and age in both the Subjects and the Controls. (Table 5)

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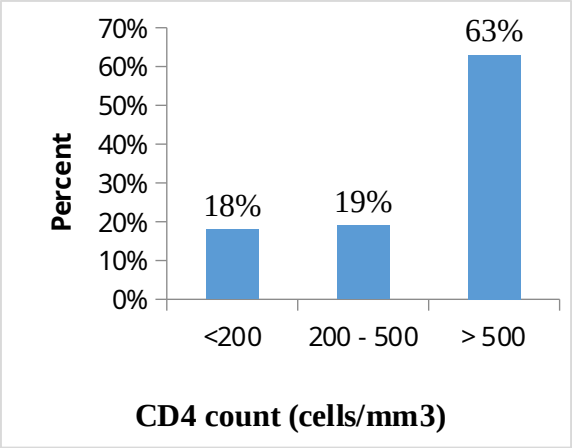

Figure 1: Prevalence of different levels of CD_4 count in the subjects

The overall minimum CD_4 count was 4 cell/mm^3 and the maximum was 2098 cells/mm^3 with a median CD4 count of 350 cells/mm^3. Eighteen percent of the study population had CD4 counts of < 200 cells. Nineteen percent of them had CD_4 counts between 200 and 500 cells. Majority of the study population (63%) had CD_4 above 500 cells. (Figure 1)

Table 6: Relationship between levels of absolute neutrophil count and CD_4 count of the Subjects

Statistics	Neutrophil counts
R (Correlation Coefficient)	0.119
R^2 (Coefficient of Determination)	0.014
B (Regression coefficient)	0.565
p value	0.239

There is no significant relationship between CD_4 count and absolute neutrophil count, p = 0.239.

Discussion

Infection with HIV ultimately leads to profound immunosuppression in which patients may present with various AIDS defining clinical conditions that may impact negatively on the quality of life of the patients. Impaired haematopoiesis, immune mediated cytopenias and altered coagulation mechanisms have all been described in HIV infected children. These abnormalities may occur as a result of HIV infection, as sequelae of HIV-related opportunistic infections or malignancies. Neutropenia and other haematological manifestations have been documented to be the second most common cause of morbidity and mortality in HIV patients.[9] A study was conducted to identify haematological parameters which could serve as pointers to the diagnosis of HIV/AIDS in resource poor settings in view of the fact that the cost of diagnostic and monitoring techniques for HIV/AIDS are unaffordable to most people in resource poor settings.[6]

This prospective observational study assessed the haematological abnormalities such as neutropenia in HIV positive ART-naïve children (subjects) and compares with the HIV negative children (controls).

The prevalence of neutropenia in this study was 18% in the subjects and 14% in the controls. This 18% prevalence was higher in studies done by Erhabor *et al.*,[12] and Amballie et al,[6] which showed prevalence of 6% and 10% respectively. The difference may be due to variation in study population, clinical conditions and study design methods.

In this study, more males had greater percentage of neutropenia than the females both in the subjects, 11(61%) Vs 7(39%) and in the controls, 9(64%) Vs 5(36%) but the differences were not statistically different.

The increase or decrease in ages of both the subjects and the controls had no effect on the neutrophil counts. This contrasted with the studies by Erhabor et al.[12] and Amballie et al.[6]

In this study, there was no significant relationship between neutropenia and CD4 counts. This was in contrast to the studies by Calenda and Chermann,[14] who reported a significant association between neutropenia and CD4 counts.

Conclusion

This study shows that there is a significant difference in neutrophil count between the HIV-positive children and their negative counterparts. It was also noted that a lower CD_4 count could not be predicted by neutropenia. It was therefore recommended that a low neutrophil count cannot be used as one of the criteria for commencing ART, especially in resource-poor climes where estimation of CD_4 count is difficult.

Conflict of interest statement: The authors report no conflicts of interest.

Print ISSN: 3043-4742 Online ISSN: 3043-4750

Funding: This study was not funded by any organization. We bore all the expenses that accrued from in study.

Acknowledgments: We are grateful to the research assistant who helped in data entering.

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Print ISSN: 3043-4742 Online ISSN: 3043-4750