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Case Study

Haematological Profile of Hiv Patients In Relation to Immune Status in Kumasi, A Case Study.

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Abstract:
Introduction: Human immunodeficiency virus (HIV) infection causes AIDS, which affects CD4 cells. The continual CD4 cells' decline leads to hematological abnormalities.
Aim: The study compared the hematological profiles to the CD4 count among HIV patients.
Materials and Method: The study was a cross-sectional study conducted from December 2018 to April 2019, involving 385 HIV positive patients who attended Anti-Retroviral Therapy (ART) at Komfo Anokye Teaching Hospital (KATH) and Obuasi Government Hospital. Five milliliters (5mls) of venous blood was collected aseptically from each participant into ethylene diamine tetra-acetic acid (EDTA) tube using disposable syringe. CD4 count (FACS caliber, Becton Dickson, Singapore) and full blood count (Sysmex XN 2000, Sysmex corporation, Japan) were performed to determine the immune status of the patients and their hematological parameters**.**
Results: Participants of the study had a mean age of 40.62 years old, mean weight of 58 kg, and had a duration of ART treatment of 6.17 years. A higher proportion of the study participants were females (73.7%), had HIV-1 (97.7%) and had lower level of formal education (83.0%).CD4 count was directly associated with haemoglobin levels (r=0.32), WBC count (r= 0.23) especially with neutrophils. However, CD4 count was negatively associated with lymphocyte count (r= -0.14).
Conclusion: There is a strong association between CD4 counts and the severity of anemia and neutropenia in HIV/AIDS patients. Anemia and neutropenia in HIV patients can be considered as good clinical indicators to predict and access the underlying immune status.

Keywords: HIV/AIDS, CD4 count, neutropenia.

Background

Human immunodeficiency virus (HIV) infection is the most common cause of acquired immune deficiency. This leads to the clinical disease being referred to as Acquired Immune Deficiency Syndrome (AIDS) (1).

HIV infects CD4 cells and in the absence of appropriate therapy leads to discerning CD4 cell lymphopenia. The progressive deterioration in CD4 cells eventually leads to the development of opportunistic infections, cancer, wasting, and death (1).

AIDS is caused by HIV which is characterized by continual damage to the body's immune system resulting in immunological and hematological complications as well as a number of opportunistic infections, (2).

Hematological abnormalities are the most common problems in HIV and these include all extractions of blood cells (3).

CD4 T lymphocyte serves as a marker for immune status. Noticeable immune compromise, as evidenced by a low CD4 lymphocyte count, is now less frequently encountered among individuals with access to HAART because most guidelines recommend treatment for individuals with CD4 lymphocyte counts below 200 cells/mm^3 and patients with CD4 lymphocyte count is less than 350 cells/mm^3 may be considered for treatment (4). According to World Health Organization (5) guidelines, preventive therapy should be started when an HIV positive person who has no symptoms registers a CD4 count under 200 cells per cubic millimeter of blood. Hematological complications among HIV patients are mostly marked with cytopenias such as anemia, neutropenia, lymphopenia and thrombocytopenia (6&7).

The severity of cytopenia and its incidence generally correlate with the stage of the disease. Here, anemia is the most occurring hematological abnormality, and a good predictor to the progression of AIDS.

Anemia and neutropenia may result from insufficient production of blood cells. This can be as a result of suppression of the bone marrow by the HIV infection through abnormal cytokine expression and alteration bone marrow microenvironment) (10).

Immune-mediated destruction of the platelets causes thrombocytopenia, in addition to the inadequate production of platelet. The stage of the disease is generally correlated to the incidence and severity of cytopenia. Other causes of cytopenia in these patients include malignancies, or other pre-existing or coexisting medical problems, treatment-related adverse events or secondary to the opportunistic infections. (6, 10 &11).

Materials and Methods

The study was a cross-sectional study from December 2018 to April 2019, and involved HIV patients who attended ART at Komfo Anokye Teaching Hospital (KATH) and Obuasi Government Hospital. Three hundred and eighty-five (385) HIV patients were randomly selected for the study. KATH is located in Kumasi, the regional capital of Ashanti region with a total population of 4,780,380 (KATH Annual Report, 2010). It is the second largest teaching Hospital after Korle Bu Teaching Hospital in the country. The geographical location of the 1200- bed capacity KATH receive referrals from all the northern regions (namely, Northern, Upper East and Upper West regions), Brong Ahafo, Central, Western, Eastern and parts of the Volta regions. Obuasi government Hospital is a district hospital located in the Obuasi Municipality. The hospital has an ART department where HIV patients are managed.

Sample Collection

Five milliliters (5mls) of venous blood was collected aseptically from each participant into ethylene diamine tetra-acetic acid (EDTA) tube using disposable syringe. The blood samples were used for full blood count (FBC) and CD4 count.

Full Blood Count Determination The full blood was determined using a hematological analyzer (sysmex XT4000i, Sysmex Japan).

Fig. 1 hematological analyzer (sysmex XT4000i Sysmex Japan).

CD4 count

CD4 count was performed by using FACS count (Becton Dickson, Singapore)

Fig. 2 FACS count (Becton Dickson Biosciences, Singapore).

CD4 counts were measured routinely in the first visit and during the follow-up visits.

Inclusion Criteria

Patients with HIV positive result were included for the study.

Exclusion Criteria

Patients with HIV negative result were excluded from the study.

Results

Table 1. Baseline Characteristics of the Study Population

Variables	Frequency (n=300)	%
	Mean ± SD	*Range*
Age (years)	40.62 ± 10.01	12-72
Weight (kg)	58.33 ± 13.40	30-119
Duration of (years)	6.17 ± 4.21	0.08-17.0
Sex		
Female	221	73.7
Male	79	26.3
Marital status		
Divorced	75	25.0
Married	128	42.7
Single	63	21.0
Widow	34	11.3
HIV type		
Type 1	293	97.7
Type 1 and 2	7	2.3
Employment status		
Formal	37	12.3
Informal	249	83.0
Unemployed	14	4.7

The mean age, weight, and duration of therapy was 40.62 years old, 58.33 kg, and 6.17 years respectively. A higher proportion of the study participants were females (73.7%), married (42.7%), had HIV-1 (97.7%),

Table 2. Haematological and immune status of the study participants

Variables	Median (Interquartile range)
Haemoglobin (g/dl)	11.55(10.16-12.80)
WBC count (10^3/µl)	4.67(3.81-5.66)
Platelet count (10^3/µl)	225.0(189.0-300.0)
Neutrophil count (10^3/µl)	1.89(1.40-2.50)
Lymphocyte count (10^3/µl)	2.40(1.80-4.08)
CD4 count (cells/µl)	378.0(185.25-632.00)

The table show the hematological and immune status of the study participants. The median hemoglobin, WBC count, platelet count, neutrophil count, lymphocyte count and CD4

count were 11.55 g/dl, 4.67x10^3/µl, 225.0x10^3/µl, 1.89x10^3/µl, 2.40x10^3/µl, and 378.0 cells/µl respectively.

Table 3. Distribution of CD4 count and haematological parameters among the study population

Variables	Frequency (n)	%
Haemoglobin		
Anaemia	201	67.0
Normal haemoglobin level	99	33.0
WBC count		
Low	137	45.7
Normal	163	54.3
Platelet count		
Low	22	7.3
Normal	268	89.3
High	10	3.3
Neutrophil count		
Low	91	30.3
Normal	203	67.7
High	6	2.0
Lymphocyte count		
Low	14	4.7
Normal	219	73.0
High	67	22.3
CD4 count		
<200	78	26.0
200-500	107	35.7
>500	115	38.3

The prevalence of anemia, leukocytopenia, and thrombocytopenia was 67.0%, 45.7%, and 7.3% respectively. Ten (3.3%) of the study participants had thrombocytosis. The proportion of study subjects with neutropenia, and lymphopenia was 30.3% and 4.7% respectively. The distribution of study subjects with CD4 count <200 cells/µl, 200-500 cells/µl, and >500 cells/µl were 26.0%, 35.7%, and 38.3%, respectively.

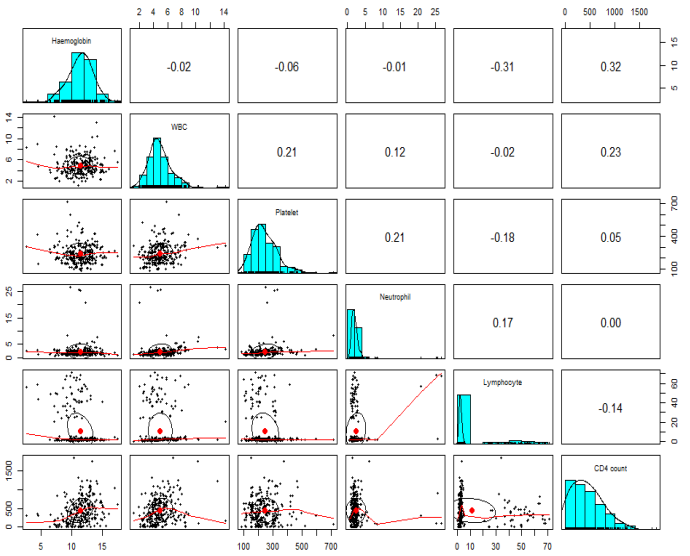
Table 4. Comparison of haematological profile by immune status

Variables	CD4 count			p-value	Sign Pairs
	<200 (a)	200-500 (b)	>500 (c)		
Haemoglobin (g/dl)	10.20(8.93-11.70)	11.50(10.40-12.80)	12.10(11.1-13.10)	<0.0001	a&b; a&c
WBC count (103/µl)	4.26(3.40-5.45)	4.70(3.80-5.80)	4.90(4.16-5.80)	0.016	a&c
Platelet count (103/µl)	224.50(195.00-300.75)	221.00(183.00-302.00)	235.00(194.00-298.00)	0.797	-
Neutrophil count (103/µl)	1.86(1.30-2.54)	1.70(1.35-2.31)	2.00(1.57-2.64)	0.040	b&c
Lymphocyte count (103/µl)	2.34(1.57-24.25)	2.47(1.80-5.38)	2.40(1.86-3.14)	0.507	-

Subjects with CD4 count <200 cells/µl presented with significantly lower hemoglobin level (10.20 g/dl) compared to those with CD4 count of 200-500 cells/µl (12.10 g/dl) and >500 cells/µl (12.1 g/dl) respectively. Additionally, subjects with CD4 count <200 cells/µl had significantly reduced WBC count (4.26x10^3/µl) compared to subjects with CD4 count > 500 cells/µl (4.90x10^3/µl), though within normal ranges. Furthermore, participants with CD4 count of 200-500 cells/µl had significantly lower neutrophil count (1.70x10^3/µl) compared to participants with CD4 count > 500 cells/µl (2.0x10^3/µl).

Figure 1. Linear association between haematological parameters and CD4 count among the study population

As shown in figure 1 below, CD4 count was directly associated with haemoglobin levels (r=0.32), WBC count (r= 0.23). However, CD4 count was negatively associated with



Discussion

Hematological complications are a common cause of mortality in HIV infected patients. Cytopenias are most frequent during the advance stage of the disease (3).

In the present study, we found out that majority of the patients were females (73.7%), married (42.7%), had HIV-1 (97.7%), and education (83.0%) and had a mean age of (40.62±10.01 years, range 12-72). Our results correlate with previous studies by Coyle, whose findings showed that, a higher proportion of the study participants were found to be in the middle and low socio- economic class, married and females. Majority of the females affected by the disease could be due to their anatomical makeup in their reproductive organ. Because the vaginal surface area is larger, it makes these females more susceptible to HIV infection than males. Married couples who assumedly would have one partner could have less probability of contracting HIV than unmarried persons, hence the low incidence of HIV infection among married persons.

Secondly, anemia, leukopenia especially neutropenia were common findings in this study. This was also documented in different studies (13, 14 &15).

The prevalence of anemia in this study (67%) was higher than study done in India in 2002 (16) and 2008 (17) which was 30.8% and 65.5% respectively, Southern India 41% (18),

Brazil 37.5% (19). This may be due to the difference in study population, socio-demographic characteristics of study subjects and study design methods.

Also, the prevalence of neutropenia in our study was (30.3%) which was slightly correlated to a study done by Attili et al., which showed that neutropenia incidence was from 13%-44%. On the other hand, prevalence of thrombocytopenia (7.3%) in this study was lower than reports from a study done by Enawgaw et al, which presented as (6.6%). This possible cause of thrombocytopenia may be due to geographical differences.

Furthermore, in our study 26.0% were cases of CD4 counts <200cells/µl which was lower than a study by Dikshit et al., which presented 92.4%. Also, 35.7% were cases of CD4 counts between 200-500cells/µl and 38.3% were cases of CD4 counts >500 cells/µl which was higher than a study by Dikshit et al., which presented 7.6% cases of CD4 counts >200cells/µl. This possibly could be due to the higher compliance to therapy of patients in Ghana and in the study, compared to those of participants of the other countries. Available of the HAART could also contribute to lower cases of HIV patients with low CD4 counts in our study

The cumulative incidence of anemia with hemoglobin levels (10.20g/dl) with a p-value (<0.0001) which is statistically significant was highest among patients who had CD4 lymphocyte count <200cells/µl and lowest among patients who had CD4 counts >500 and 200- 500cells/µl with hemoglobin levels of 12.1g/dl in our study which agrees to a work done by Dikshit et al where the incidence of anemia was higher in patients with CD4 counts <200cells/µl. and lower in those with CD4 counts >500cells/µl. The similarity could be attributed to the fact that anemia is a major indicator to the HIV disease when lymphocyte counts when low. This trend was recorded in the study by Dikshit et al

Individuals having HIV and infected with anemia are at risk for progression to AIDS and mortality, while recovery from anemia has been associated with a decreased risk of deaths.

Moreover, patients with CD4 count <200 cells/µl had significantly reduced WBC count ($4.26x10^3$/µl) compared to patients with CD4 count > 500 cells/µl ($4.90x10^3$/µl), though within normal ranges. Previous studies done by Shen et al., suggested that there is increase WBC count with decreased CD4 count. Furthermore, participants with CD4 count of 200-500 cells/µl had significantly lower neutrophil count ($1.70x10^3$/µl) compared to participants with CD4 count > 500 cells/µl ($2.0x10^3$/µl) which does not agree with work done by Attili et al., which shown that the incidence of neutropenia was 0.8% in HIV patients with CD4 count >700cell/ml.

Though a fall in the CD4 levels during neutropenia is observed, it is difficult to comment as the estimations of CD4 rely on the total leukocyte count.

We also found out that platelet count of 224.50×10^3/µl ,221.00×10^3/µl and 235×10^3/µl compared to CD4 count were < 200, 200-500 and >500cells/µl respectively. Lymphocyte counts of 2.34×10^3/µl, 2.47×10^3/µl and 2.40×10^3/µl compared to CD4counts were < 200, 200-500 and >500cells/µl respectively. p-values of platelets and lymphocyte were (0.797) and (0.507) respectively which are statistically insignificant. Platelet and lymphocyte counts were not directly correlated to the stage of the disease. We therefore saw that the increase in the stage of the disease did not necessarily decrease lymphocyte counts or affect the trend of increase or decrease in platelet count.

In our study, CD4 count was directly associated with hemoglobin levels (r=0.32), WBC count (r= 0.23) which is not in agreement with a work done by Venkata et al., where there was a strong negative association between CD4 count and anemia and neutropenia. It is unclear why Venkata et al.,

Surprisingly, CD4 count was negatively associated with lymphocyte count (r= -0.14). This may be due to the measurement of only CD4 count with the exclusion of CD3 and CD8.

Conclusion

This study revealed that

1. there is a strong association between CD4 counts and the severity of anemia and neutropenia in HIV/AIDS patients.
2. Anemia and neutropenia in HIV patients can be considered as good clinical indicators to predict and access the underlying immune status.
3. The relation between anemia and disease progression is straightforward and quite useful for the treatment of patients.
4. It can also help hospitals without equipment's for CD4 counts to estimate the levels of CD4 cells using the hemoglobin level and absolute neutrophil count of the patients, only in the management of the progression or otherwise of the patient for HIV into AIDS

Recommendation

1. Policy makers of the health sector are to encourage HIV patients to check up their CD4 count monthly
2. Policy makers can also enforce that hematological parameters should be performed for every patient on each visit particularly with the early detection stages of the disease
3. Policy makers should ensure the early start of HAART when it is appropriate in order to reduce the prevalence of anemia and neutropenia.

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