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ORIGINAL RESEARCH

Hematological abnormalities in HIV-antiretroviral therapy naïve clients as seen at an immune suppression syndrome clinic at Mbarara Regional Referral Hospital, southwestern Uganda

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Aim/objective: To assess the common hematological abnormalities among HIV-antiretroviral therapy (ART) naïve clients attending an immune suppression syndrome (ISS) clinic at Mbarara Regional Referral Hospital (MRRH), southwestern Uganda.

Patients and methods: This was a cross-sectional study carried out during the months of March to August 2016 at the ISS clinic of MRRH. We collected approximately 4.0 mL of EDTA anticoagulated blood samples, which were assayed for complete blood count, CD4+ cell count and thin film examination. Correlation of the hematological abnormalities with CD4+ cell counts was done using correlation coefficient (r) and analysis of variance (F), and the p-value was set at ≤0.05.

Results: A total of 141 clients were enrolled. Of these, 67.38% (95/141) were anemic, 26.24% (40/141) had thrombocytopenia while 26.95% (38/141) had leucopenia. Of the 95 participants with anemia, 89.47% (85/95) presented with normocytic-normochromic anemia, 8.42% (8/95) with microcytic-hypochromic anemia and 2.11% (2/95) with macrocytic-hypochromic anemia. Anemia was not different across the several World Health Organization (WHO) stages of HIV infection disease progression ($p>0.05$). Statistically significant differences were present among participants with leucopenia ($p<0.05$). Also, leucopenia was more prevalent (11/38) among participants in WHO stage 4 of HIV infection. CD4+ cell counts correlated with thrombocytopenia ($r=0.24$, $p<0.05$) and leucopenia ($r=0.15$, $p<0.05$).

Conclusion: People living with HIV/AIDS (PLWHIV/AIDS) ought to be routinely monitored and treated for the occurrence of hematological abnormalities. Early initiation of ART can help to prevent some hematological abnormalities.

Keywords: antiretroviral therapy, HIV, leucopenia, anemia, thrombocytopenia, Uganda

Introduction

According to the Uganda AIDS Commission (UAC), HIV remains a threat, as evidenced by the prevalence rate increasing from 6.4% to 7.3%.[1] Consequently, some of the HIV-infected individual will progress to the AIDS state, which is a systemic disorder characterized by severe impairment and progressive damage of both cellular and humoral immune responses. Over time, hematological abnormalities have been documented as independent predictors of morbidity and mortality among HIV-infected individuals.[2,3] As part of routine monitoring and diagnosis, the use of cluster of differentiation (CD), especially CD4-T lymphocyte marker and full blood count, has been vital as pre-treatment investigations for people living with HIV/AIDS (PLWHIV/

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AIDS).[4,5] This has demonstrated changes in blood cells, which are indicative of the effect of HIV infection.[5] Although they are neither part of the criteria for initiating therapy, nor used by the World Health Organization (WHO) for staging HIV, hematological abnormalities indicated by deranged full blood count are common manifestations and important prognostic tools of HIV infection and AIDS.[6] Anemia, leucopenia and thrombocytopenia are common during the course of HIV infection, and may be due to the direct effects of HIV infection, secondary infections or side effects of anti-retroviral therapy.[7]

Cytopenias are common in the advanced stages of AIDS and often caused by concomitant myelosuppressive drugs. Although hematological abnormalities are common in HIV infected clients and have great effect on their well-being and treatment, few studies on this have been undertaken in Uganda. Thus, we report on the prevalence and pattern of hematological abnormalities in HIV antiretroviral therapy (ART) naïve clients at an immune suppression syndrome (ISS) clinic of Mbarara Regional Referral Hospital (MRRH), southwestern Uganda.

Patients and methods

Study participants

The participants were HIV sero-positive clients enrolled in the ISS clinic, MRRH, located within Mbarara Municipality, Mbarara district in southwestern Uganda.

Sample collection

Approximately 4 mL of blood was drawn by venipuncture from study participants into an EDTA vacutainer.

Laboratory analysis

Full blood count and CD4+ cell counts were done using the Coulter ACT 5Diff CP and Facscalibur, as per standard operating procedures at the ISS clinic. Reference ranges as determined for the HIV population were used.[8] Thrombocytopenia was regarded as platelet count less than 150×10^9/L, anemia was defined as hemoglobin concentration less than 12 g/L for adult females and less than 13 g/dL for adult males, and leucopenia was considered as white blood cell (WBC) count less than 2.75×10^9/L. Thin blood films were prepared, stained by Giemsa and examined for anemia typing based on: a) size of red blood cells (normocytic, microcytic and macrocytic) and b) hemoglobin content (normochromic and hypochromic). Pancytopenia was considered if all the three blood cells were below their minimum for a reference range. We ensured strict adherence to the standard operating procedures, the hematology analyzer was well maintained and controls were run daily before use. A hematology bench aid was used to ascertain cell features of a comprehensive film comment.

Statistical analysis

Descriptive statistics (mean, median, inter-quartile range and standard deviation) were used. The chi-square test, analysis of variance and *Z* tests were used to establish predictor variables. Proportions and percentages were calculated for categorical variables, and were compared using 95% confidence interval. Correlation of the hematological abnormalities with CD4+ cell counts was done using correlation coefficient (r) and analysis of variance (F). The p-value was set at ≤ 0.05.

Ethical considerations

We obtained ethical approval from Mbarara University of Science and Technology Faculty of Medicine Research and Ethics Committee (FREC), and the university's Research and Ethics Committee (REC). All participants provided written informed consent.

Results

A total of 141 HIV positive ART naïve participants were enrolled. This was a cohort of advanced HIV disease with the overwhelming majority of participants with CD4 cells below 350. They comprised 56.03% (n=79) males. Their mean age was 34 years (95% CI=33, 36 years); the minimum and maximum age was 17 and 63 years respectively, with a median age of 32 years (interquartile range [IQR] 28, 42 years). Their socio-demographic, hematological and immunologic features are presented in Table 1.

Of the 141 participants, 67.38% (95% CI=58.98–75.03) were anemic, 26.24% (95% CI=19.19–34.31) had thrombocytopenia while 26.95% (95% CI=19.83–35.07) had leucopenia. The pattern of cytopenias was as follows: 62 (43.97%; 95% CI=40.54–58.68) had anemia alone, 17 (12.06%; 95% CI=8.13–20.88) had both anemia and thrombocytopenia while 11 (7.80%; 95% CI=4.48–15.2) had both anemia and leucopenia. Eight (5.67%; 95% CI=2.80–12.22) had thrombocytopenia alone, 7 (4.96%; 95% CI=2.28–11.19) had both thrombocytopenia and leucopenia while 15 (10.64%; 95% CI=6.87–19.02) had leucopenia alone. There were 5 (3.54%; 95% CI=1.31–9.09) participants who had pancytopenia.

Table 1 Demographic, hematologic and immunologic characteristics of study participants (n=141)

Variables	Category	Frequency	Percentage
Age (years)	18–27	33	23.40
	28–37	52	36.88
	38–47	41	29.08
	48–57	12	8.51
	58 and above	3	2.13
Gender	Male	79	56.03
	Female	62	43.97
CD4+ cell counts (/L)	0–349	139	98.58
	350–499	0	0.00
	Above 500	2	1.42
WHO clinical stage	Stage I	10	7.19
	Stage II	50	35.97
	Stage III	65	46.76
	Stage I–4	14	10.07
RBC total ($\times 10^{12}$/L)	0–3.7	63	44.68
	3.8–5.9	78	55.32
	Above 5.9	0	0.00
Hb (g/dL)	0.0–5.0	0	0.00
	5.1–8.0	11	7.80
	8.1–11.9	84	59.57
	Above 11.9	46	32.62
WBC total ($\times 10^9$/L)	0.0–3.5	70	49.65
	3.6–11.0	68	48.23
	Above 11.0	3	2.13

Abbreviations: Hb, hemoglobin; RBC, red blood cell; WHO, World Health Organization; WBC, white blood cell.

Thin film examination showed most participants to have normocytic-normochromic anemia (85/95; 89.47%), then microcytic-hypochromic anemia (8/95; 8.42%) and least had macrocytic-hypochromic anemia (2/95; 2.11%). Microcytic-hypochromic anemia was more likely to occur (8/10; 80%) compared to normocytic-normochromic anemia (85/122; 69.67%) and macrocytic-hypochromic anemia (2/9; 22.22%) (χ^2=9.37, p=0.009). Leucopenia was more associated with neutropenia (χ^2=37.14; p<0.001) compared to lymphopenia (χ^2=18.44; p<0.001). For platelet counts, 86.49%, (95% CI=71.23–95.46) had mild thrombocytopenia, while 3.51% (95% CI=4.54–28.74) had severe thrombocytopenia, as indicated in Table 2.

WHO clinical staging indicated no difference in the prevalence of anemia and thrombocytopenia among all the clinical stages (Z=–1.23, p=0.22 and Z=2.74, p=0.06 respectively). Leucopenia was more among participants in clinical stage 4 (Z=2.08, p=0.03). The details are shown in Table 3.

Correlation of the common hematological abnormalities with CD4+ cell counts showed that diminished CD4+ cell counts had no association with anemia (r=–0.0002), but the association exists with thrombocytopenia and leucopenia (r=0.24, and 0.15 respectively) as given in Table 4.

Table 2 Distribution of cytopenias (n=125)

Cytopenias	Anemia	Thrombocytopenia	Leukopenia	Pancytopenia
Anemia	62 (49.6%; 95%CI 40.54–58.68)			
Thrombocytopenia	17 (13.6%; 95% CI 8.13–20.88)	8 (6.4%; 95% CI 2.8–12.22)		
Leukopenia	11 (8.8%; 95% CI 4.48–15.2)	7 (5.6%; 95% CI 2.28–11.19)	15 (12%; 95% CI 6.87–19.02)	
Pancytopenia				5 (4%; 95% CI 1.31–9.09)

Table 3 Hematological abnormalities at each WHO HIV stage

Cytopenias	WHO stage				*p*-value
	1	2	3	4	
Anemia	7 (70%)	31 (60.78%)	48 (72.73%)	9 (64.29%)	0.22
Leucopenia	1 (10%)	6 (11.76%)	20 (30.3%)	11 (78.57%)	0.03
Thrombocytopenia	0 (0%)	7 (13.73%)	23 (34.84%)	7 (50%)	0.06

Abbreviation: WHO, World Health Organization.

Table 4 Correlation of hematological abnormalities with CD4+ cell count

Hematological parameters	CD4 cell categories		*r*	*F*	*p*-value
	≥200 cells/μL (n=13)	<200 cells/μL (n=128)			
Hb (mean±SD)	11.1 (±0.18)	10.63 (±0.57)	–0.0002	0.63	0.430
Platelets (mean±SD)	207.23 (±8.96)	307.46 (±28.12)	0.24	11.53	<0.001
Total leucocyte count (mean±SD)	3.68 (±0.21)	5.18 (±0.67)	0.15	4.54	0.030

Notes: *F*, analysis of variance; *r*, correlation coefficient.
Abbreviation: Hb, hemoglobin.

Discussion

Cytopenias have been widely reported among people living with HIV/AIDS.

Anemia was the most prevalent cytopenia, accounting for 67.38% (95% CI=58.98–75.03). This is similar to the 64.2% reported in India,[9] although lower than the 82.4% in a review.[10] The high prevalence is explained by the effect of myelodyplasia on the erythroid cells.[9,11–13]

Morphological characterization of anemia based on comprehensive film comment indicated normocytic-normochromic (85/95; 89.47%), microcytic-hypochromic (8/95; 8.42%) and macrocytic-hypochromic (2/95; 2.11%) types. Normocytic-normochromic anemia suggested continued bone marrow myelodyplasia.[9,11] This occurs as a result of decreased ability of bone marrow to synthesize and release blood cells into circulation, a phenomenon that is evident in HIV infection, as well anti-retroviral therapy.[11] On the other hand, the variant (microcytic-hypochromic and macrocytic hypochromic) forms of anemia are nutrient deficiency indicators.[14–17] Nutrient deficiencies are common in clients with HIV immunosuppression, mainly due to the impact of anorexia, medication associated gastrointestinal disturbances, wasting and malabsorption.[17] In some cases, iron deficiency anemia secondary to HIV, related to gastrointestinal blood loss has been reported.[15–17] This is the probable explanation for the occurrence of microcytosis in our study. The etiology of vitamin B12 deficiency in these patients has been linked to malabsorption in the distal ileum and an alteration in cobalamin transport proteins.[14] Folate deficiency can occur in HIV-infected patients and may result from reductions in both dietary intake and intestinal absorption,[14] which explains the occurrence of macrocytic anemia among our study participants.

Thrombocytopenia accounted for 26.24% (95% CI=19.19–34.31). This was similar to what was found in Chinese adults.[18] On the other hand, results from this study were higher than the values of 8.3% and 17.8% reported in Uganda,[19,20] and 5.9% in Ethiopia.[2] The pathogenesis of thrombocytopenia has been associated with immune causes,[18,20,21] which arise early in the course of HIV infection but are slightly more prevalent in those with advanced disease.[21]

In this study, 26.95% (95% CI=19.83–0.07%) of the participants had leucopenia, results similar to the values of 26.8% and 26.6% found in Nigeria.[5,22] In this study, leucopenia was more associated with neutropenia (χ^2=37.14; p<0.001). This phenomenon is ascribed to the autoimmune destruction as the pathogenesis of neutropenia. In addition, HIV is a likely mediator of defective hematopoiesis through mechanisms like direct infection of early hemopoetic precursors, aberrations of local cytokine and growth factor signaling and changes in the bone marrow stroma.[3] As there is limited literature about demographic indicators of neutropenia, we attribute leucopenia to be the likely cause of neutropenia, as reported earlier.[3] Pancytopenia was seen in 3.54%, and this is attributed to opportunistic infections including Mycobacteria and fungi (including *Pneumocystis carinii*, *Crypococcus neformas* and *Penicillium marneffei*) that are capable of broad dissemination throughout the bone marrow, directly affecting the progenitor cells.[3]

There was no difference in the prevalence of anemia and thrombocytopenia among all the WHO HIV clinical stages, but a difference occurred within the WBC population. Leucopenia was more common among participants in clinical stage 4 (Z=2.08, p=0.03). The fact that HIV affects the CD4+ and natural killer cells which are part of the population of WBCs can explain why we found leucopenia more in the advanced clinical stages (Z=2.08, p=0.03).[9,11,23]

A correlation of the common hematological abnormalities with CD4+ cell counts indicated that deteriorating immune status had no statistical association with anemia (r=–0.0002), which was similar to other studies.[24–26] Besides immunological exposures, there are other causes of anemia in HIV infection, e.g. autoimmune antibodies to hemopoetic precursors and opportunistic infections like parvovirus B19, cytomegalovirus (CMV), or *Mycobacterium avium-intracellulare* which suppress erythropoiesis. Also hemolytic anemia, gastrointestinal bleeding and malnutrition with folate, vitamin B12 and iron have been reported to cause anemia in HIV infection.[27]

A slight association exists with thrombocytopenia and leucopenia (r=0.24 and 0.15, respectively) to decreasing CD4+ cell counts. This was similar to results from Ethiopia.[2] As already elucidated, thrombocytopenia is associated with advanced AIDS.[20] Since CD4+ cell count is used as a feature of deteriorating HIV infection, this may explain the correlation between thrombocytopenia and lower CD4+ cell counts. Thus, there is a need to prevent CD4+ cell counts decreasing, which is achieved by early initiation of ART, and routine monitoring of the effectiveness of the different regimen.

The results of this study should be interpreted with the following limitations in mind: a) we did not investigate the risk of cytopenia due to opportunistic infections, yet this population was highly susceptible; b) we did not investigate the possibility of hemoglobinopathies as likely causes of anemia; c) although we attribute nutrient deficiency as a probable

cause of nutritional anemia, we did not assay for iron, vitamin B12 and folate and d) we did not study the bone marrow, thus we are lacking the bone marrow hemopoetic picture.

Conclusion

We report a high risk of cytopenias (anemia, leucopenia and thrombocytopenia) among PLWHIV. The study has also found that a decrease in the CD4+ cell counts had no association with anemia, but an association exists with thrombocytopenia and leucopenia. Also, the occurrence of anemia and thrombocytopenia was not associated with any clinical stage, but those in clinical stage 4 were more likely to have leucopenia. With the high risk of cytopenias, it is imperative to initiate PLWHIV/AIDS on ART early, and routinely monitoring the response.

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Author contributions

CK, IMT, CA, EM, CM and BM participated in study conception and design, data acquisition, analysis and interpretation, manuscript drafting and revising. CA, BM and IMT critically revised the manuscript. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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