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Frequency of common hematological manifestations determined in newly diagnosed HIV infected patients

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Abstract

Globally the phenomenon of Human immunodeficiency virus (HIV) and *acquired immune deficiency syndrome* (AIDS) is noticed as pandemic, which affects almost all countries around the globe. Presently in Asia, about 4.9 million population are infected with HIV. It attacks the immune system of human body. Generally, the immune system generates several infection counter cells called T-cell lymphocytes. After infecting the immune system, within months to years, all the T-cell lymphocytes get destroy. Current study was aimed to determine the frequency of common hematological manifestations in newly diagnosed HIV infected patients. In this study, total of 271 patients were observed by using prevalence of neutropenia 2.29%, 95% confidence interval and 2% margin error using WHO sample size calculator. Moreover, non-probability consecutive sampling was used to collect samples. In this study, the mean age was 32 ± 3.51 years. 62%percent patients were male and 38% patients were female. Hematological manifestations were analyzed as 80% patients had anemia, 38% patients had total leucocyte count < 4000/µL, 20% patients had absolute lymphocyte count < 800/µL, 5% patients had absolute neutrophil count < 1000 /µL while 15% patients had total platelet count <150,000. Our study concludes that the most common hematological manifestations was anemia 80% followed by total leucocyte count < 4000/µL (38%), absolute lymphocyte count < 800/µL (20%), total platelet count <150,000 (15%) and absolute neutrophil count < 1000 /µL (5%).

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Introduction

Body's immune system get attack by HIV. Basically, the immune system generates certain fighting cells against infection called T-cell lymphocytes. After infection by HIV, within months to years the virus damage the entire T-cell lymphocytes. This terminates the capability of the immune system to further protect the body against infection and tumors. Several opportunistic infections take full advantage of such weak immune system and antibiotic resistance is quite obvious among HIV infected people (Malik *et al.*, 2017).

Several infections that are normal in healthy body, than cause severe health issues that eventually lead the HIV patient towards death (Imtiaz, 2014).Basically, HIV infection affects several systems and causes multisystem disease, with hematological abnormalities amongst the most common clinicopathological manifestations of HIV infection (Wanjari *et al.*, 2013; Shen *et al.*, 2013; Imtiaz, 2014). Hematological abnormalities have been documented as strong predictors of mortality and morbidity in HIV infected patients (Shen *et al.*, 2013).Hematological complications among HIV are generally marked with cytopenias such as anemia, thrombocytopenia, leucopenia, and lymphopenia.The occurrence and severity of the cytopenias normally correlate with the stage of disease (Ogba *et al.*, 2013).

Hematological problems are more common during advanced HIV infection and can severely affect the highly active anti-retroviral therapy (HAART) outcomes, causing in higher mortality (Choi *et al.*, 2011). It has been observed that CD4 lymphocyte counts, World Health Organization clinical stage, anemia status, body weight, advanced age, total lymphocyte count, and male sex are have more mortality rate in sub-Saharan Africa. Moreover country locality and ethnicity can also affect hematological manifestations in HIV patients (Choi *et al.*, 2011).Several secondary factors such as the medications effect, hepatitis B virus(HBV) and hepatitis C coinfection, opportunistic infections, and liver cirrhosis also contribute to hematological manifestations (Choi *et al.*, 2011).

The anemia prevalence within HIV patients widely varies by sex and race/ethnicity (Prendushi *et al.*, 2014). Anemia as the most common in patients infected with HIV has been associated with impaired quality of life, accelerated progression of disease, and a higher mortality rate (Prendushi *et al.*, 2014).Previous studies demonstrated that HIV patients and patients of low level hemoglobin have a more mortality risks compared to less anemic groups even after controlling both CD4 cell count and viral load. Anemia is associated with thrombocytopenia and neutropenia because of myelosuppression (Prendushi *et al.*, 2014). Anemia is the most common hematological problem in HIV infected patients (Choi *et al.*, 2011; Ogba *et al.*, 2013; Prendushi *et al.*, 2014). In one cross-sectional retrospective study carried out in 131 patients 82.4% had anemia (Prendushi *et al.*, 2014).

The prevalence of anemia was 83.3% in female patients and 82.1% in males. 89% of patients had anemia in the low CD4 group (less than 200) and 52.9% in the high CD4 group (more than 200).

Lymphopenia was noted in 63.4%, neutropenia in 2.29%, and thrombocytopenia in 6.1% of the patients (Prendushi *et al.*, 2014).Another prospective cross sectional study done in 100 confirmed cases of HIV/AIDS which showed that microcytic hypochromic anemia was seen in 73%, 19% had total TLC of < 4000 cells/µL. 62% subjects had CD4 count <200/mm(Imtiaz, 2014). 12% subjects had CD4 counts < 50 cells/mm (Wanjari *et al.*, 2013).

While going through literature search it became evident that hematological manifestations are common among HIV patients across the globe due to increasing burden of the disease in developing countries, but they vary from one population to another in terms of ethnicity and demographics (Choi *et al.*, 2011).

Also a very few data is available about our

community. So the rationale behind our study is to determine the frequency of common hematological manifestations among new HIV/AIDS patients so that the reversible factors (like iron deficiency, nutritional deficiency anemia due to folic acid and vitamin B12 deficiency, parasitic infestations or blood loss) are identified and treated before the commencement of antiretroviral
therapy (ART) and study design was cross sectional.

Materials and methods

This study was accomplished in Department of Medicine, Lady Reading Hospital Peshawar, Pakistan. Study duration was 6 months (9/12/2014 to 10/5/2015).Sample size was 271 patients, with prevalence of neutropenia 2.29% (Prendushi *et al*., 2014), 95% confidence interval and 2% margin error using WHO sample size calculator. Sampling technique was non probability consecutive sampling.

Sample selection

Inclusion criteria

Inclusion criteria of patients for this study was; (1) All male and female patients with HIV infection according to operational definition e.g. patients who are HIV positive by ELISA and according to WHO classification having(i) Primary HIV infection: either asymptomatic or associated with acute retroviral syndrome; (ii) STAGE 1: Asymptomatic with CD4 count more than 500/µL or generalized lymphadenopathy; (iii) STAGE 2: Minor mucocutaneous symptoms and recurrent respiratory tract infections.(2) Age between 15-60 years (3) patients not on Anti-Retroviral therapy.

Exclusion criteria

The exclusion criteria included (1) Stage 3 and stage 4 disease (2) Causes of anemia, leucopenia and thrombocytopenia due to other acute or chronic medical illness (3) Patients with any hereditary or previous acquired hematological disorder (4) Patients on any drug causing blood dyscrasias.

Data Collection Process

The study was carried out after getting approval from hospital ethical and research committee. All patients meeting the inclusion criteria and presenting with HIV (as per operational definition above) were enrolled in the study through out-patient department, emergency and /or clinic of the consultants working currently in medical C department of Lady Reading Hospital, patients were admitted in the hospital if required. Written informed consent from all included patients was taken and were explained the objectives of the study. Confidentiality of the patients was maintained.

All included patients had detailed history and detailed clinical examination. After inclusion blood sample was taken in EDTA TUBE for detection of anemia, leucopenia, neutropenia, lymphopenia and thrombocytopenia by hematology automatic analyzer (SYSMEX KX-21 and SYSMEX XS -1000) currently available.

All the laboratory investigations was done from hospital laboratory and hematology section under supervision of a single hematologist having minimum of five years of experience. CD4 T-lymphocyte count was done by flow-cytometry that is available free of cost in Family Care Center (FCC) Hayatabad Medical Complex Peshawar, Pakistan.

All information included name, gender, age, and address recorded in a proforma. Exclusion criteria had strictly followed to control confounders and bias in the study results.

Data analysis

Data was entered and analyzed in SPSS version 17. Mean ± Standard Deviation was calculated for numerical variables like age and CD4 count. Frequencies and percentages were calculated for gender, anemia, leukopenia, neutropenia, lymphopenia and thrombocytopenia. Hematological abnormalities were stratified among age and gender to see the effect modifications. Post stratification was applied through chi-square test, keeping p- value ≤ 0.05 was considered significant. All the results were presented as tables.

Results

This study was conducted at Department of Medicine, Lady Reading Hospital Peshawar, Pakistan in which 271 patients were observed to determine the frequency of common hematological manifestations in newly diagnosed HIV infected patients. Age-wise distribution of 271 patients was analyzed and the highest number was of 103(38%) patients were in age range 31-40 years while the least one was 52(19%) patients were in age range 51-60 years.

Details of age distribution among patients are given in (Table 1). Mean age was 32 ± 3.51 years.

Table 1. Age-wise distribution of patients (n=271).

Age	Frequency	Percentage
18-30 years	62	23%
31-40 years	103	38%
41-50 years	54	20%
51-60 years	52	19%
Total	271	100%

Gender-wise distribution among 271 patients was analyzed as 168(62%) patients were male while 103(38%) patients were female. Among patients, 163 (60%) haveCD4 count range from 500 - 600/µL, while 108 (40%) have700 - 800/µL. Mean CD4 count was 570 ± 2.71/µL. Hematological manifestations among 271 patients was analyzed, as 216(80%) patients had anemia, 103(38%) patients had total leucocyte count <4000/µL, 54(20%) patients had absolute lymphocyte count <800/ µL, 14(5%) patients had absolute neutrophil count <1000 / µL while 41(15%) patients had total platelet count <150,000 (Table 2).

Table 2. Hematological manifestations (n=271).

Hematological manifestations	Frequency	Percentage
Anemia	216	80%
Total leucocyte count <4000/UL	103	38%
Absolute lymphocyte count <800/UL	54	20%
Absolute neutrophil count <1000 /UL	14	5%
Total platelet count <150,000	41	15%

Stratification of each hematological manifestations with respect to age and gender is given in Tables 3 - 12.

Only stratification of anemia with respect to age-wise distribution was significant ($P < 0.05$), while all others were not significant ($P > 0.05$).

Discussion

Hematological problems are common in patients with advanced HIV infection and can affect the outcomes of highly active anti-retroviral therapy (HAART), resulting in higher mortality (Choi*et al.*, 2011).

It has been observed that CD4 lymphocyte counts, World Health Organization clinical stage, anemia status, body weight, total lymphocyte count, advanced age, and male sex are more related to mortality in sub-Saharan Africa.

Moreover country locality and ethnicity can also affect hematological manifestations. Several secondary factors such as medications effect, hepatitis B virus(HBV) and hepatitis C coinfection,

opportunistic infections, and liver cirrhosis also contribute to hematological manifestations (Choi*et al.*, 2011).

Our study shows that mean age was 32± 3.51 years. 62% patients were male while 38% patients were female. Hematological manifestations was analyzed as 80% patients had anemia, 38% patients had total leucocyte count <4000/µL, 20% patients had absolute lymphocyte count <800/µL, 5% patients had absolute neutrophil count <1000 /µLwhile 15% patients had total platelet count <150,000.

In one cross-sectional retrospective study carried out in 131 patients 82.4% had anemia (Prendushi*et al.,* 2014).

The prevalence of anemia was 83.3% in female patients and 82.1% in males. 89% of patients had anemia in the low CD4 group (less than 200) and 52.9% in the high CD4 group (more than 200).

Lymphopenia was noted in 63.4%, neutropenia in 2.29%, and thrombocytopenia in 6.1% of the patients (Prendushi*et al.,* 2014).

Table 3. Stratification of anemia with respect to age distribution (n=217).

Age	Anemia		Total
	Yes	No	
18-30 years	30	32	62
31-40 years	82	21	103
41-50 years	53	1	54
51-60 years	51	1	52
Total	216	55	271
Legends	Chi square test was applied (P = 0.0001)		

Table 4. Stratification of anemia with respect to gender distribution (n=217).

Age	Anemia		Total
	Yes	No	
Male	134	34	168
Female	82	21	103
Total	216	55	271
Legend	Chi square test was applied (P = 0.9761)		

Another prospective cross sectional study done in 100 confirmed cases of HIV/AIDS which showed that microcytic hypochromic anemia was seen in 73%, 19% had total TLC of < 4000 cells/µL. 62% subjects had CD4 count < 200/mm(Imtiaz, 2014). 12% subjects had CD4 counts < 50 cells/mm (Wanjari *et al.*, 2013).While going through literature search it became evident that hematological manifestations are common among HIV patients across the globe due to increasing burden of the disease in developing countries, but they vary from one population to another in terms of ethnicity and demographics (Choi *et al.*, 2011).

Table 5. Stratification of total Leucocyte count < 4000/µL with respect to age-wise distribution(n=217).

Age	Total leucocyte count <4000/µL		Total
	Yes	No	
18-30 years	24	38	62
31-40 years	38	65	103
41-50 years	21	33	54
51-60 years	20	32	52
Total	103	168	271
Legend	Chi square test was applied (P = 0.9930)		

Also a very few data is available about our community. So the rationale behind our study is to determine the frequency of common hematological manifestations among new HIV/AIDS patients so that the reversible factors (like iron deficiency, nutritional deficiency anemia due to folic acid and vitamin B12 deficiency, parasitic infestations or blood loss) are identified and treated before the commencement of antiretroviral therapy (ART).

Our results were in line with another study carried out by Addis *et al.* (2014)in which the prevalence of immune-hematological abnormalities of HIV positive individuals during their first visit to the ART clinic. Accordingly 19% and 12.2% of the study participants had leucopenia and thrombocytopenia respectively. Anemia was observed among 42.3% of the study participants. Leucopenia and lymphopenia were observed among 4.8% and 12.7% of the study participants.

Table 6. Stratification of total Leucocyte count< 4000/µL with respect to gender distribution (n=217).

Age	Total leucocyte count< 4000/µL		Total
	Yes	No	
Male	64	104	168
Female	39	64	103
Total	103	168	271
Legend	Chi square test was applied (P = 0.9696)		

Table 7. Stratification of absolute Lymphocyte count< 800/µL with respect to age-wise distribution (n=217).

Age	Absolute lymphocyte count <800/µL		Total
	Yes	No	
18-30 years	12	50	62
31-40 years	21	82	103
41-50 years	11	43	54
51-60 years	10	42	52
Total	54	217	271
Legend	Chi square test was applied (P = 0.9971)		

The most common abnormality observed was immune-suppression (CD4 count < 500 cells/mm^3) which is found in 83.1% of the individuals, majority, 43.7%, being severely immune-suppressed. The prevalence of anemia in this study was 80% which is higher than a study conducted in India that showed a prevalence of 65.5% from a total of 200 study participants (Dikshit *et al.*, 2009). A study from Ghana has also reported anemia prevalence of 63.5% among 206 HAART naïve patients which is higher than the current report (Owiredu *et al.*, 2011).

Table 8. Stratification of absolute Lymphocyte count< 800/µL with respect to gender-wise distribution (n=217).

Age	Absolute lymphocyte count<800/µL		Total
	Yes	No	
Male	33	135	168
Female	21	82	103
Total	54	217	271
Legend	Chi square test was applied (P = 0.8814)		

On the other hand studies from Iran and Nigeria reported a prevalence of 10.3% from 642 individuals and 24.2% from 205 individuals (Jam *et al.*, 2009; Akinbami *et al.*, 2010) which are lower than the current report. Differences observed may be attributed to the difference in the demographic

profile, sample size difference and variability in the definition of anemia. A relatively similar result was reported from the study area among HAART naïve patients (Ferede and Wondimeneh, 2013).

The anemia prevalence among female participants was 22.2% in our study and this was similar with a study from Rwanda that reported a prevalence of 20.5% (Munyazesa *et al.*, 2012). In this study prevalence of anemia was not significantly associated with different characteristics of the study participants which is different from other studies where associations with some factors like sex and CD4 count were reported(Jam *et al.*, 2009; Ferede and Wondimeneh, 2013).

Table 9. Stratification of absolute Neutrophil count< 1000 /µL with respect to age-wise distribution (n=217).

Age	Absolute neutrophil count<1000 /µL		Total
	Yes	No	
18-30 years	3	59	62
31-40 years	5	98	103
41-50 years	3	51	54
51-60 years	3	49	52
Total	14	257	271
Legend	Chi square test was applied (P = 0.9930)		

Table 10. Stratification of absolute Neutrophil count< 1000 /µL with respect to gender-wise distribution (n=217).

Age	Absolute neutrophil count <1000 /µL		Total
	Yes	No	
Male	9	159	168
Female	5	98	103
Total	14	257	271
Legend	Chi square test was applied (P = 0.8559)		

The prevalence of leucopenia in this study was 38% which is higher than a follow up study conducted in Nigeria that reported a prevalence of 26.8% (Ibeh *et al.*, 2013). On the other hand a lower prevalence, of 5.5%, was also reported (Denue*et al.*, 2013). In this study high rate of leucopenia was significantly associated with immune-suppression (low CD4 count) as reported elsewhere in the world (Zon *et al.*, 1987; Calenda and Chermann, 1992). About 43.9% of the study participants were severely immuno compromised (had a CD4 count of less than 200 cells/µL) requiring immediate initiation of ART as per the WHO guideline (WHO; 2005).

Table 11. Stratification of total platelet count < 150,000 with respect to age-wise distribution (n=217).

Age	Total platelet count<150,000		Total
	Yes	No	
18-30 years	9	53	62
31-40 years	16	87	103
41-50 years	8	46	54
51-60 years	8	44	52
Total	41	230	271
Legend	Chi square test was applied (P = 0.9980)		

Lymphopenia was observed in about 20% of the study participants which was similar to a report by Ogba *et al.* (2013). About 5% of the study participants had neutropenia and are hence at great risk of developing infections at different sites including the skin, mucosa and the lungs (Boxer, 2012). In the current study, thrombocytopenia was reported among 12.2% of the study participants. This result is higher than a previous report in the study area that showed a prevalence of 5.9% (Wondimeneh *et al.*, 2014).

Table 12. Stratification of total platelet count <150,000 with respect to gender distribution (n=217).

Age	Total platelet count <150,000		Total
	Yes	No	
Male	25	143	168
Female	16	87	103
Total	41	230	271
Legend	Chi square test was applied (P = 0.8842).		

An Indian study has also reported a lower prevalence (4.65%) of thrombocytopenia (Mathews *et al.*, 2013). Higher results in our study may be due the difference in the study population. Because the current study participants were newly diagnosed HIV positive individuals who might had visited the ART clinic after the disease has progressed to a severe state that will increase the prevalence of thrombocytopenia. A thrombocytopenia prevalence of 20% was reported from Iran which higher than our report (Alaei *et al.*, 2002).

The low cut of value used to define thrombocytopenia in the Iranian study may contribute to higher prevalence rates. Similar to the study conducted by Wondimeneh *etal.*(2014) neither the socio demographic factors nor the CD4 count of the study participants showed significant association with the prevalence of thrombocytopenia.

In another study more than one fourth (30%) of the patients had more than one hematological abnormality and 13.2% had cytopenia. The result is lower than a report from Nigeria that indicated an overall cytopenia prevalence of 20% (Akinbami *et al.*, 2010). Though, the result seems smaller as compared to the Nigerian study, it indicates that large numbers of HIV positive individuals are at great risk for HIV associated morbidity and mortality as the larger population is considered. Hence, great attention should be given to this group of individuals and the various reasons behind the cytopenia need to be investigated for better management of the patients.

Conclusion

The hematological abnormalities observed in this study are very high, especially the rate of immune-suppression and anemia. This high prevalence may be associated with delayed visit to ART providing institutions. Hence, the association of health seeking behavior or HIV positive patients and the state of hematological manifestations need to be understood. Moreover further research that will assess the clinical profile of HIV positive patients in the first visit to the ART centers and their association with hematological profile need to be researched as it will provide more important information for the care and management of these patients.

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