

HIV-associated thrombocytopenia: Profiling hematological changes in the HIV infected and AIDS patients

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Abstract

Background and Aim: The first Acquired Immune Deficiency Syndrome (AIDS) case in India was detected in the year 1986. Seldom studies have been conducted correlating Cluster of Differentiation-4 cell counts (CD4 cell counts) and complete blood picture including platelet counts in the Human Immunodeficiency Virus (HIV) infected and AIDS patients in the Indian population. The present study was carried-out with the same intent to evaluate the CD4 cell counts and complete blood picture including platelet counts in the HIV infected and AIDS patients and correlate them with the sero-negative controls.

Materials and Methods: The present study was a cross-sectional, hospital-based study on the HIV infected and AIDS patients. Evaluation of complete blood picture including platelet counts was done using Sysmex XP 100- (Sysmex Corp., Hyogo, Japan), a fully automated analyzer while CD4 cell counts were evaluated using Partec Cyflow Counter- (Partec GmbH, Münster, Germany).

Statistical Analysis Used: The data was analyzed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Comparison of the said parameters was done using Analysis of Variance (ANOVA) and post-hoc Games-Howell test. p-value of <0.05 was considered statistically significant.

Results: The levels of hemoglobin (Hb), white cell counts (WBCs) and platelet counts showed statistically significant results ($p < 0.001$) with the levels of Hb and platelet counts significantly decreased and levels of WBCs significantly increased in the HIV infected and AIDS groups as against the sero-negative controls.

Conclusion: The levels of hemoglobin (Hb), white cell counts (WBCs) and platelet counts were significantly altered in the HIV infected and AIDS groups when compared with the sero-negative controls.

Key words: CD4 cell counts, Complete blood picture, Platelet counts, HIV, AIDS

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Introduction

AIDS is an Acronym For Acquired Immune Deficiency Syndrome caused by a retrovirus known as human immunodeficiency virus (HIV) which breaks down the body's immune system leaving the patient vulnerable to a host of life threatening opportunistic infections, neurological disorders or, unusual malignancies [1]. The two known types of this virus include the HIV-1 and HIV-2 which belong to a family of primate lentiviruses [2,3]. According

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to estimates by World Health Organization (WHO) and The Joint United Nations Programme on HIV/AIDS (UNAIDS), 35 million people were living with HIV globally at the end of the year 2013 [4]. The first AIDS case in India was detected in the year 1986 [4]. HIV is transmitted by both homosexual and heterosexual contact, by blood and blood products, by infected mothers to infants either via intra-partum or, peri-natal routes or, via breast milk and by occupational transmission [5].

There are two major targets of HIV infection: the immune system and the central nervous system. Profound immuno-suppression, primarily affecting the cell mediated immunity (CMI), is the hallmark of AIDS. HIV enters the body through mucosal tissues and blood and first infects the T cells as well as dendritic cells and macrophages. The infection becomes established in lymphoid tissue where the virus may remain latent for long periods. Active viral replication is associated with more infection of cells and progression to AIDS. In addition to the lymphoid tissue, the nervous system is a major target of HIV infection. Macrophages and microglia cells in the central nervous system that belong to the monocyte and macrophage lineage are the predominant cell types in the brain that are infected with HIV [3].

India carries the third largest number of HIV infected and AIDS patients in the world after South Africa and Nigeria [6]. In India, the highest prevalence of HIV/AIDS cases has been observed in Nagaland followed by Mizoram, Manipur and Andhra Pradesh according to the latest national AIDS statistics by National AIDS Control Organization (NACO, HIV Sentinel Surveillance 2012-13) [7]. HIV infection causes depletion of CD4 cells in peripheral blood and lymphoid tissues causing CD8 cell dysfunction. Quantification of CD4 helper lymphocytes is, thus, essential in the staging and monitoring of HIV infected and AIDS patients. With reduced CD4 cell counts in HIV infection, granulocytopenia and thrombocytopenia are seen. When the counts of granulocytes fall < 500 per mm^3 , in the presence of an attendant anatomical barrier damage that follows the viral infection, invasion of the bloodstream by microorganisms is facilitated with resultant sepsis and death. The CD4+T lymphocytes are the primary target of HIV infection because of the affinity of the virus to the CD4+ cell surface marker. Infection with HIV leads to a progressive impairment of cellular functions characterized by a gradual decline of CD4+T lymphocyte levels in peripheral bloodstream which results in an increasing susceptibility to a wide variety of opportunistic, viral, bacterial, protozoal, and fungal infections and also, to certain malignancies [1,3].

Haematological abnormalities are amongst the most common complications of infection with HIV [8]. Chronic thrombocytopenia develops in approximately one third of individuals infected with the HIV during the course of the acquired immunodeficiency syndrome [9]. Different studies have been carried-out on CD4 cell counts and hematologic parameters in the HIV infected and AIDS patients in different parts of the world including Nigeria, Brazil, Thailand, Switzerland and Ghana. In India, few studies were reported from Uttar Pradesh, Chandigarh, Karnataka, Tamil Nadu and Manipur. Seldom studies have been conducted correlating CD4 cell counts and complete blood picture including platelet counts in HIV infected and AIDS patients in the Indian population. The present study was

carried-out with the same intent to evaluate the CD4 cell counts and complete blood picture including platelet counts in the HIV infected and AIDS patients and correlate them with the sero-negative controls.

Materials and Methods

The present study was designed as a cross-sectional, hospital-based study to assess CD4 cell counts and complete blood picture including platelet counts in the HIV infected and AIDS patients and correlates them with the sero-negative controls. The study population included 1500 subjects reporting to the Outpatient Department who were divided into 3 groups including:

Control Group: consisting of 500 individuals who were healthy controls without any systemic illness;

HIV Group: consisting of 500 patients who were diagnosed as HIV infected; and

AIDS Group: consisting of 500 patients diagnosed as AIDS patients, depending on their CD4 cell counts.

The permission from the Ethics Committee of the Institution was obtained before the start of the study. Also, an informed consent was obtained from the patients forming the study sample to participate in the study to analyze their CD4 cell counts and complete blood picture including platelet counts. The patients at the extremes of ages, pregnant women and those on chemotherapy were excluded from the study because of possible weakened immune status. The patients who did not agree to give consent and were not willing to participate in the study were, also, excluded from the study. The patients were made to sit in the chair comfortably and a detailed history was taken followed by the clinical examination which was performed following the protocols of the Universal Precautions on each subject in the ART Centre with the help of diagnostic instruments and artificial illumination. The findings were recorded in a specialized proforma while all the patients were, then, subjected to phlebotomy procedure.

Phlebotomy procedure: The patient was explained about the procedure. The patient's forearm was rested on the laboratory table comfortably. The ante-cubital fossa was exposed and the tourniquet was applied about half an inch to two inches above the ante-cubital fossa. The area was rendered aseptic with 70 % ethyl alcohol and using a sterile disposable syringe and 23-gauge needle, a needle puncture was made and maneuvered to enter the ante-cubital vein and 2 ml of blood was drawn. The tourniquet was, then, relieved and the needle was removed. Dry cotton was placed on the site of needle puncture on the forearm and instructions were given to apply finger pressure for about five minutes and dispose the cotton. The blood was transferred immediately into the tubes containing EDTA.

Evaluation of CD4 cell counts in the HIV infected and AIDS Patients: For this, 50 μ l of Ethylene-diamine-tetraacetic acid (EDTA) anti-coagulated blood was added to 10 μ l of monoclonal antibody and after 15 minutes of incubation, 1 ml of No Lyse dilution buffer was added and the sample tube was attached to the Partec Cyflow Counter- (Partec GmbH, Münster, Germany) (**Figure 1**) for an automated evaluation of CD4 cell counts in the collected samples.

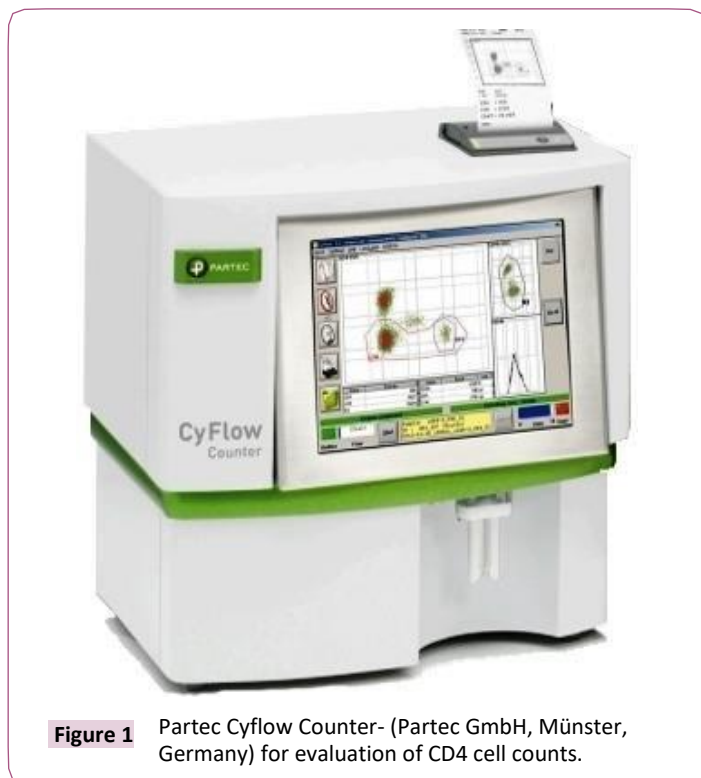


Figure 1 Partec Cyflow Counter- (Partec GmbH, Münster, Germany) for evaluation of CD4 cell counts.

Evaluation of complete blood picture including platelet counts in the HIV infected and AIDS patients: Complete blood picture was obtained using Sysmex XP 100- (Sysmex Corp., Hyogo, Japan) (Figure 2), a compact, fully automated 3 part differential hematology analyzer. For evaluation of complete blood picture including platelet counts in the study and control groups, 50 μ l of blood was taken as the sample in a 2 step procedure. The automated analyzer sampled the blood and quantified, classified and described the cell populations using both electrical and optical techniques. Electrical analysis involved passing a dilute solution of the blood through an aperture across which an electric current was flowing. The passage of cells through the current changed the impedance between the terminals. A lytic reagent was added to the blood solution to selectively lyse the red blood cells (RBCs) leaving only the white blood cells (WBCs) and platelets intact. The solution was, then, passed through a second detector. This allowed the counts of RBCs, WBCs and platelets to be obtained. The platelets were easily separated from the WBCs by the smaller impedance spikes they produced in the detector due to their lower cell volumes. Similarly, optical detection was, also, utilized to gain differential counts of WBCs. A dilute suspension of cells was, then, passed through a flow cell which passed cells one at a time through a capillary tube past a laser beam. The reflectance, transmission and scattering of the light from each cell were analyzed by sophisticated software giving a numerical representation of the likely overall distribution of the cell populations.

Statistical Analysis Used: The data was analyzed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Comparison of the said parameters was done using Analysis of Variance (ANOVA) and post-hoc Games-Howell test. p-value of <0.05 was considered statistically significant.

Result

The present study was designed as a cross-sectional, hospital-based study to assess CD4 cell counts and complete blood picture including platelet counts in the HIV infected and AIDS patients and correlate them with the sero-negative controls. The distribution of patients based on age and gender as well as the distribution of male and female patients based on age is shown in [Tables.1-3]. In the present study, the mean CD4 cell count in the control group was found to be 1125.38 while in the HIV group, it was seen to be 501.35 and in AIDS group, 256.41 dropping down significantly with the p-value being <0.001 as the HIV infection progressed to full blown AIDS [Table 4 and Graph 1]. Furthermore, on analyzing the complete blood picture including platelet counts in the said patients, a mean Hb of 13.75 was observed in the control group while a mean of 13.38 was observed in the HIV group and 12.37 in the AIDS group. The results were found to be statistically significant in this case, too, with the p-value being <0.001 [Table 5 and Graph 2]. The variations in the mean PCV observed in the present study, though, were found to be conflicting with a mean PCV of 37.88 observed in the control group with a mean of 38.23 in the HIV group and 37.63 in the AIDS group. The results, therefore, in this case, were not found to be statistically significant [Table 5]. Likewise, in case of RBCs, the variations in the values observed in the controls, the HIV and the AIDS groups were found to be conflicting and the results, too, were found to be statistically insignificant with a mean RBC count of 4.59 observed in the control group while 4.57 in the HIV and 4.64 in the AIDS groups [Table 5]. The observations made in case of WBC counts, though, were found to be statistically significant in the present study with a mean WBC count of 8134.84 observed in the control group while a mean of 9688.40 in the HIV and 10264.00 in the AIDS groups. The results, in this case, were found to be statistically significant with the p-value being <0.001 [Table 5 and Graph 3]. The present study had a notable finding of thrombocytopenia detected with statistically significant results in the HIV infected and AIDS patients with a mean platelet count of 3.37 observed in the control group while 3.21 in the HIV group which dropped down significantly to a mean platelet count of 2.92 in the AIDS group [Table 5 and Graph 4]. To summarize, The levels of hemoglobin (Hb), white cell counts (WBCs) and platelet



Figure 2 Sysmex XP 100- (Sysmex Corp., Hyogo, Japan) for evaluation of complete blood picture including platelet counts.

Table 1. Distribution of patients based on age groups.

| Age group (in years) | Control Group | % | HIV Group | % | AIDS Group | % |
|----------------------|---------------|-------|-----------|-------|------------|-------|
| 10 – 20 | 40 | 8% | 31 | 6.2% | 16 | 3.2% |
| 21 – 30 | 127 | 25.4% | 193 | 38.6% | 150 | 30% |
| 31 – 40 | 99 | 19.8% | 161 | 32.2% | 181 | 36.2% |
| 41 – 50 | 126 | 25.2% | 79 | 15.8% | 102 | 20.4% |
| 51 – 60 | 73 | 14.6% | 21 | 4.2% | 38 | 7.6% |
| 61 – 70 | 35 | 7% | 15 | 3% | 13 | 2.6% |

Table 2. Distribution of patients based on gender.

| Gender | Control Group | % | HIV Group | % | AIDS Group | % |
|---------|---------------|-------|-----------|-----|------------|-------|
| Males | 291 | 58.2% | 235 | 47% | 259 | 51.8% |
| Females | 209 | 41.8% | 265 | 53% | 241 | 48.2% |

Table 3. Distribution of male and female patients based on age groups.

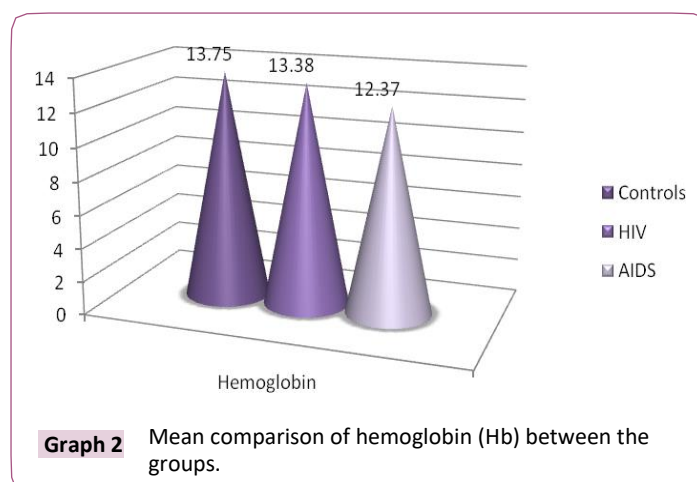
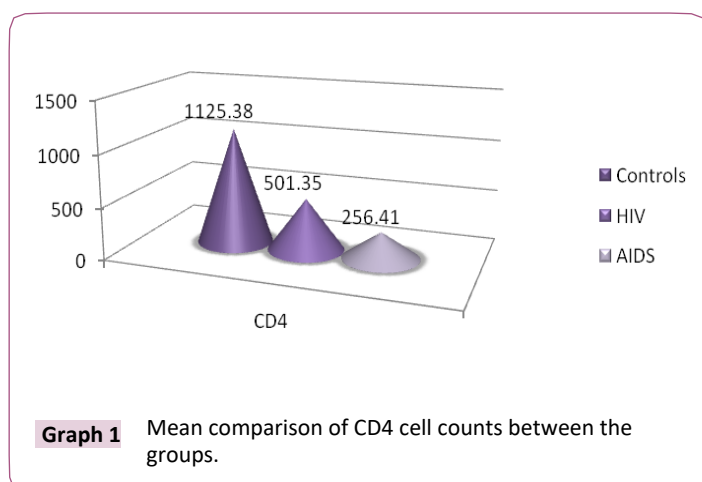
| Age group (in years) | Control Group | | | | HIV Group | | | | AIDS Group | | | |
|----------------------|---------------|-------|--------|------|-----------|-------|--------|-------|------------|------|--------|-------|
| | Male | % | Female | % | Male | % | Female | % | Male | % | Female | % |
| 10 – 20 | 29 | 5.8% | 11 | 2.2% | 11 | 2.2% | 20 | 4% | 06 | 1.2% | 10 | 2% |
| 21 – 30 | 79 | 15.8% | 48 | 9.6% | 79 | 15.8% | 114 | 22.8% | 70 | 14% | 80 | 16% |
| 31 – 40 | 52 | 10.4% | 47 | 9.4% | 81 | 16.2% | 80 | 16% | 90 | 18% | 91 | 18.2% |
| 41 – 50 | 71 | 14.2% | 55 | 11% | 41 | 8.2% | 38 | 7.6% | 55 | 11% | 47 | 9.4% |
| 51 – 60 | 38 | 7.6% | 35 | 7% | 14 | 2.8% | 07 | 1.4% | 31 | 6.2% | 07 | 1.4% |
| 61 – 70 | 22 | 4.4% | 13 | 2.6% | 09 | 1.8% | 06 | 1.2% | 07 | 1.4% | 06 | 1.2% |

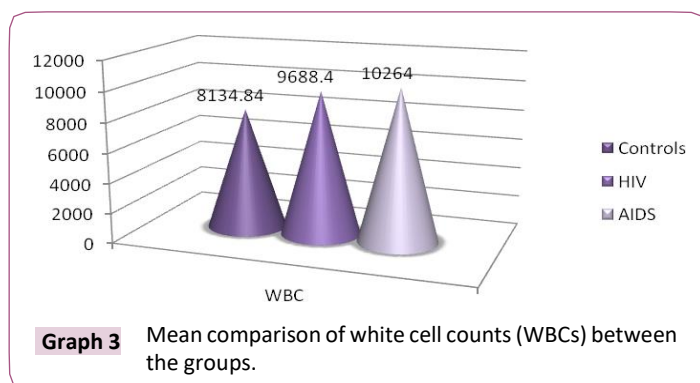
Table 4. Evaluation of CD4 cell counts in the three groups (p-value <0.001 Statistically significant).

| Control Group | | HIV Group | | AIDS Group | | p-value | Post-hoc test |
|---------------|--------|-----------|--------|------------|-------|---------|---------------|
| Mean | SD | Mean | SD | Mean | SD | | |
| 1125.38 | 154.73 | 501.35 | 140.20 | 256.41 | 67.05 | <0.001 | C>H>A |

Table 5. Evaluation of complete blood picture including platelet counts in the three groups (p-value <0.001 Statistically significant).

| | Control Group | | HIV Group | | AIDS Group | | p-value | Post-hoc test |
|--------------------------|---------------|---------|-----------|---------|------------|---------|---------|---------------|
| | Mean | SD | Mean | SD | Mean | SD | | |
| Hemoglobin (Hb) | 13.75 | 1.76 | 13.38 | 1.87 | 12.37 | 1.18 | <0.001 | C>H>A |
| Packed cell volume (PCV) | 37.88 | 3.18 | 38.23 | 21.24 | 37.63 | 5.46 | 0.614 | - |
| Red cell counts (RBCs) | 4.59 | 0.43 | 4.57 | 0.74 | 4.64 | 0.73 | 0.334 | - |
| White cell counts (WBCs) | 8134.84 | 3988.69 | 9688.40 | 2813.78 | 10264.00 | 5819.57 | <0.001 | H,A>C |
| Platelet counts | 3.37 | 0.66 | 3.21 | 0.64 | 2.92 | 1.91 | <0.001 | C>H>A |





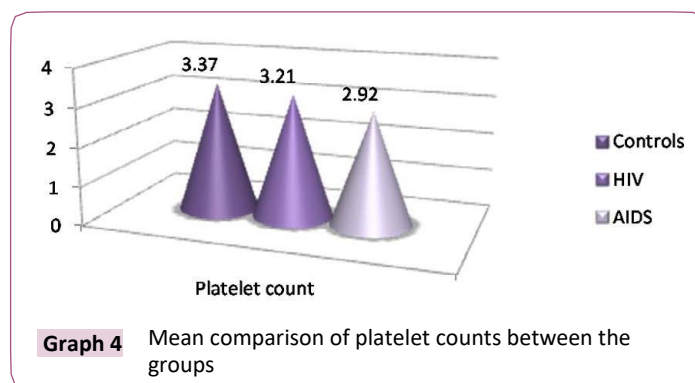
Graph 3 Mean comparison of white cell counts (WBCs) between the groups.

counts showed statistically significant results ($p < 0.001$) with the levels of Hb and platelet counts significantly decreased and levels of WBCs significantly increased in the HIV infected and AIDS groups as against the sero-negative controls. [Table 5 and Graphs.2-4].

Discussion

CD4 cell counts is essential for assessment of immune status in HIV infected individuals as the pathogenesis of AIDS is largely attributed to a decrease in absolute CD4 cell counts [10]. Different methods have been implemented in evaluating the CD4 cell counts by different authors [11]. used Coulter manual CD4 kit for evaluating the CD4 cell counts [12]. estimated the CD4 cell counts by using a formula where total leucocyte count was multiplied by lymphocyte % age and divided by 100 and then, multiplied by 100th part of CD4 % age [9,13]. estimated the CD4 cell counts by using Fluorescence Activated Cell Sorter (FACS) [14]. estimated the CD4 cell counts using automated flow cytometer software (multi-set) [15]. estimated the CD4 cell counts using Flow Cytometry Absolute Cell Count System at NPHL [16]. estimated the CD4 cell counts using conventional flow cytometry using a Becton-Dickinson FACScount [17]. estimated the CD4 cell counts using FACS Counter [18]. estimated CD4 cell counts by standard flow cytometry using FACS Calibur. Pranitha SS and Kulkarni MH10 estimated CD4 cell counts in BD FACS Calibur flow cytometer, an automated multi-color system. In the present study, Partec Cyflow Counter- (Partec GmbH, Münster, Germany) was used to estimate the CD4 cell counts as it was relatively small, reputed to be easy to use and had a high throughput of samples.

In the present study, the mean CD4 cell count in the control group was found to be 1125.38 while in the HIV group, it was seen to be 501.35 and in AIDS group, 256.41 dropping down significantly with the p -value being < 0.001 as the HIV infection progressed to full blown AIDS. The gradual decrease in the CD4 cell counts observed in the HIV infected and AIDS patients in the present study when compared to the controls were still higher than the mean values observed in the studies conducted by Pasupathi and Saravanan [9,19] who recorded a mean CD4 cell count of 394 in the HIV infected and 191 in the AIDS groups and 375 in the HIV infected and 150 in the AIDS groups respectively in two different studies although the results obtained were found to be in accordance with the results of the study conducted by Tiwari [15] who recorded a mean value of 281 cells per mm^3 in



Graph 4 Mean comparison of platelet counts between the groups

the HIV patients. The reason for the higher values obtained in the present study than as compared to most of the studies might be due to the difference in the classification of the patients into HIV infected and AIDS patients based on the CD4 cell counts. In the present study, the HIV infected and AIDS patients were categorized based on their CD4 cell counts with 10-350 and 350-500 cells per mm^3 of blood.

The CD4 lymphocytes are the primary target of HIV infection because of the affinity of the virus to the CD4 cell surface receptors (CD4+). Infection with HIV leads to a progressive impairment of cellular functions characterized by a gradual decline in peripheral blood CD4+ lymphocyte levels which results in an increasing susceptibility to a wide variety of opportunistic viral, bacterial, protozoal and fungal infections and certain malignancies [15]. reported that the CD4 cell counts decreased in HIV infection due to the disruption of the cell membranes of the said cells brought-out by the budding of the infecting virus from the surface of the cells as well as the intra-cellular accumulation of the hetero-disperse RNAs and un-integrated DNAs with the progression of the disease process. Furthermore, it has, also, been proposed that an intra-cellular complexing of CD4 cells with the viral envelope products results in cell killing. Similarly, [15] proposed untimely induction of a programmed cell death (apoptosis) as an additional mechanism for CD4 cell loss in HIV infection.

Different methods have been implemented to evaluate the hematologic parameters by various authors [20]. estimated the RBC, WBC and platelet counts and haemoglobin using fully automated haematology analyzer (Pentra- XL 80) and observed significant decrease in the RBC and platelet counts and hemoglobin while significant increase in the WBC counts in the AIDS group compared to the HIV infected group and sero-negative controls [21]. estimated blood cell counts by using an ABX Pentra 120 DX automated hematology analyzer [17]. estimated blood cell counts by haematology analyzer and hemoglobin by cyanmethemoglobin method. Daniel Nii Aryee Tagoe and Evelyn Asantewaa [22], also, estimated the RBC, WBC and platelet counts and hemoglobin by automated blood analyzer and observed significant decrease in the RBC and platelet counts and hemoglobin while significant increase in the WBC counts in the HIV positive than negative subjects. Pranitha SS and Kulkarni MH10 estimated the hematology parameters by using an auto-analyzer and observed significant increase in the WBC counts and significant decrease in the platelet counts in AIDS group when compared to the HIV infected and sero-negative controls. In the

present study, Sysmex XP 100- (Sysmex Corp., Hyogo, Japan) automated analyzer was used for the evaluation of complete blood picture including platelet counts as it has been said to be more reliable, accurate and less time taking than other methods.

The results of the present study were found to be in accordance with the results of the studies conducted by Manivannan and Dora Mbanya [9,16] who observed decreased levels of RBC counts, platelets and hemoglobin and increased levels of WBC counts in the HIV infected and AIDS patients in their study. The mean value of 12.37 of hemoglobin in the AIDS patients in the present study was in close accordance with the mean haemoglobin level of 11.34 reported in the study conducted by Treacy [23], while slightly higher than the mean hemoglobin of 10.20 as reported in the study conducted by Pranitha SS and Kulkarni MH10, 10.20 in the study conducted by Daniel Nii Aryee Tagoe and Evelyn Asantewaa22 and 10.8 in the study conducted by Kaloutsi [24]. The low levels of hemoglobin as well as the RBC counts might be a result of decreased red blood cell production and/or, ineffective erythropoiesis seen in the HIV infected and AIDS patients.

Thrombocytopenia observed in the present study, too, was in accordance with the results reported in the study conducted by Erhabor [25]. The degree of thrombocytopenia was, also, found to be directly related to the degree of immunosuppression as was confirmed in the study conducted by Jost and Akinbami [26-29] also, reported the prevalence of thrombocytopenia in their respective studies. According to Pranitha SS and Kulkarni MH10, the mechanism of thrombocytopenia in HIV infection appears to involve increased platelet destruction and ineffective platelet production. Most reports indicate that there is significant platelet sequestration and destruction in the spleen in HIV associated thrombocytopenia. Platelet destruction is predominant early in the course of the disease process while in the later stages, decreased platelet production is assumed to be the major cause of thrombocytopenia observed in the HIV infected and AIDS patients. Possible mechanisms for thrombocytopenia as hypothesized in various studies, also, include increased platelet destruction caused either by the non-specific deposition of Circulating Immune Complexes (CICs) on platelets or, by the presence of specific anti-platelet antibodies directed against the platelets and/or, a direct infection of megakaryocytes by the HIV with a subsequent decrease in platelet production.

The results of the present study were, also, found to be in accordance with the results of the study conducted by Costello [30] who reported anemia, thrombocytopenia and various permutations in majority of the HIV infected patients. According to the results obtained by Walsh and Harbol [31-33], chronic thrombocytopenia develops in approximately one third of the individuals infected with the human immunodeficiency virus during the course of AIDS. The said finding was, though, contradictory to the results of the study conducted by Daniel [22] who observed higher platelet counts in the HIV group compared to the sero-negative controls.

HIV infection is associated with a wide variety of hematological changes as a result of bone marrow defects and immune cytopenia directly resulting from the HIV infection, opportunistic

infections or, lymphoma as well as seen as the side effects of the drugs used to treat HIV itself or, the compounding infections and/or, lymphomas. The result of bone marrow defect targeting individual hematological parameters usually leads to severe changes in the profile of these patients. Additionally, HIV induced destruction of CD4+ lymphocytes which regulate cellular and humoral immunity by interacting with other T lymphocytes, B lymphocytes, macrophages and natural killer (NK) cells does result in variations in the WBC counts with associated increased infections in these set of patients.

Conclusion

The levels of hemoglobin (Hb), white cell counts (WBCs) and platelet counts were significantly altered in the HIV infected and AIDS groups when compared with the sero-negative controls. Further studies are, however, mandated from across the country with correlation analyses to come to valid conclusions and manage this deadly, infectious disease process.

Limitations of the present study

The major limitation of the present study was that duration of the disease was not recorded in the present study apart from elicitation of any co-existing disease process which could have been significant in affecting the haematological changes seen in the present study. Also, the present study did not take into consideration the pre-ART and ART patients since the present study was not a longitudinal study where in-patient follow-up could be done.

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